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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: NIELSEN=3A

In re Application of:	)	Art Unit:
	)	
NIELSEN et al.	)	Examiner:
	)	
Appln. No.: 09/845,716	)	Washington, D.C.
	)	
Filed: May 2, 2001	)	August 1, 2001
	)	
For: TREATMENT OF ALPHA-MSH...	)	

REQUEST FOR PRIORITY

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with the provisions of 37 C.F.R. §1.55 and the requirements of 35 U.S.C. §119, there is filed herewith the certified copies of:

Appln. No.: PA 2000 01204	**Filed: August 11, 2000.**
Appln. No.: PA 2000 01757	**Filed: November 22, 2000.**
Appln. No.: PA 2001 00369	**Filed: March 6, 2001.**

It is respectfully requested that applicant be granted the benefit of the priority date of the foreign application.

Respectfully submitted,

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Attorneys for Applicant(s)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
Soren NIELSEN et al	)	Examiner:
Appln. No.: Not yet assigned	)	Washington, D.C.
Entered: May 2, 2001	)	May 9, 2001
For: TREATMENT OF ALPHA-MSH OR EPOETIN SEPARATELY …	)	Docket No.: NIELSEN3A

ASSERTION OF FOREIGN PRIORITY

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicants hereby assert priority under 35USC§119, from the following prior foreign applications:

Denmark PA 2000 01204	11 August 2000
Denmark PA 2000 01757	22 November 2000
Denmark PA 2001 00369	6 March 2001

Certified copies will be filed in due course.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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Kongeriget Danmark

Patent application No.: PA 2000 01204

Date of filing: 11 August 2000

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This is to certify the correctness of the following information:

The attached photocopy is a true copy of the following document:

- The specification, claims and figures as filed with the application on the filing date indicated above.

Patent- og Varemærkestyrelsen • Kongeriget Danmark •

Patent- og
Varemærkestyrelsen
Erhvervsministeriet

Taastrup 13 June 2001

Karin Schlichting
Head Clerk

Methods for treatment of non-ischemic kidn y diseases and diseases of th urinary tract

The present invention relates to a method for treatment or prevention of a non-ischemia condition in one or more organ(s) or tissue(s) of the urinary tract system including the kidney and the prostata comprising administration of i) an effective dosage of α-MSH and/or of an α-MSH equivalent or of ii) EPO and/or of an equivalent of EPO; or in a preferred embodiment, an combination thereof to the individual in need thereof. The invention also relates to pharmaceutical composition including a kit comprising the combination.

According to the present invention it has been shown that alpha-MSH, EPO either alone or en combination is useful for treatment of conditions of the urinary tract system including the prostata. In this respect, 1) single compound treatment (either alpha-MSH or EPO) of ureteral obstruction, nephritic syndrome and interstitial cystitis being non-ischemic conditions, and 2) combination treatment with alpha-MSH analogs and epoetin-alpha of ureteral obstruction, nephritic syndrome and cystitis has a marked effect which is significantly better than single compound treatments.

Brief description of the invention

The effect of treatment with i.v. α-MSH analogs alone, epoetin alone or α-MSH analogs and epoetin combined was established in models of non-ischemic kidney diseases and diseases of the urinary tract including the bladder. Models include ureteral obstruction, aminoglycoside induced nephrotic syndrome and interstitial cystitis. Various functional parameters were determined and the expression levels of relevant transporters were monitored to establish the effect of these compounds in these settings.

Temporal ureteral obstruction for 24 hours (uni or bilateral) followed by release of obstruction for various time periods (1-30 days) induced significant changes in kidney and urinary tract function. Markers include functional parameters, downregulation of renal water and sodium transporters. Treatment with i.v. α-MSH -analoges alone, epoetin alone or α-MSH and epoetin combined markedly reduced the downregulation of the renal marker proteins and prevented the reduction in kidney and urinary tract function.

In rat models of purimycin or adriamycin induced nephrotic syndrome acites and proteinuria developed as prominent signs of severe nephritic syndrome. Other markers included downregulation of renal water channels (aquaporins) and sodium transporters. Treatment with i.v. alfa-MSH-analoges alone, epoetin alone or α-MSH and epoetin combined markedly reduced the downregulation of the renal marker proteins and reduced proteinuria and acites production. In models of acute heart failure (temporary ischemia) or temporal intestinal ischemia combined alfa-MSH and epoetin treatment had a dramatical effect far superior of treatment with any of the components alone.

Models of interstitial cystitis in guinea pigs revealed that single alpha-MSH or EPO treatment or combined treatment with EPO plus alpha-MSH markedly reduced bladder hyperreactivity, decreased bladder eosinophil counts and other inflammatory parameters.

Detailed description of the invention.

The present invention relates to a method for treatment or prevention of an non-ischemia condition in one or more organ(s) or tissue(s) of the urinary tract system including the kidney and the prostata. The method comprises the administration of an effective dosage of α-MSH and/or of an α-MSH equivalent to the individual in need thereof. In another embodiment the invention relates to the administration of a EPO or EPO equivalent and in a still further and preferred embodiment the invention relates to the use of both EPO and α-MSH in the treatment. In the present context, the EPO, the α-MSH as well as the combination each represent an embodiment of the invention and the treatments may be changed individually if no other is specified. Accordingly, the term pharmaceutical may represent the either α-MSH and/or of α-MSH equivalent, EPO and/or EPO equivalent as well as any combination thereof. (An example is α-MSH together with a EPO equivalent).

One preferred target organ is the kidney, including tubules and glomeruli. However, alså other cell types may be present in the urinary tract system including the kidney and the prostata which thereby is the subject for treament according to the invention.
These tissues may accordingly comprise one or more cell types selected from macrophages, the reticulo endothelial system monocytes, neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, T-cells, B-cells, mast cells, and dendritic cells4. Method according to claim 3 wherein the cell type is selected from T-cells, B-cells, and mast cells, .

The dosage of the pharmaceutical in question may administered profylactically for preventing a progress of the condition, or of any symptom of the condition. It may be administered profylactically for preventing the establishment of the condition or of any symptom of the condition. The pharmaceutically active may be administered as a single dosage, as continued administration including a regimen where specific dosages are precribed for a shorter or longer duration, or as a sequential administration similarly with many treatment schedules for cancer therapy.

The origin of the condition may include anatomic abnormality of the tissue or organ; conditions caused by a chemical trauma including drugs such as adriamycin and other cancer drugs; electromagnetic radiation; renal and/or ureteric calculi, especially when the calculi occur frequently.

In addition, an infection infections by by virus, bacteria, fungus and including AIDS virus, bacterial septicemia, systemic fungal infections, Rickettsial diseases, toxic shock syndrome, infectious mononucleosis, cytomegalovirus infection, cambylobacter, salmonella, influenza, poliomyelitis, toxoplasmosis, Lassa Fever, Yellow Fever, billharziose, colibacteria, enterococcer, preteus, klebsiella, pseudomonas, staphylococcus aureus, staphylococcus epidermidis, candida albicans, and tuberculosis may be responsible for the condition to be treated.

Many cancer or by premalignant a disorders of the urinary tract system including the kidney and the prostata may cause complications which can be treated according to the present invention. Accordingly, the present invention relates to treatment or prevention of a condtion is caused by a cancer or a by premalignant disorder having an impact of the unrinary tract system including the kidney and the prostata, such as acute leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, myeloma, metastasing carcinoma of any origin.

The preferred target organ according to the invention is ureteres, kidney, bladder, urethra and the prostate gland and wherein the tissue(s) is selected from the group of lymphoid tissues, mucosa, epithelium, and endothelium. However also other parts of the organs may be succesfully treated according to the invention.

A very interesting embodiment relates to the treatment and/or prevention of cystitis, specially interstitiel cystitis, but also cystitis limited to the mucosa. Th cystitis may be a microbial cystitis or a non-microbial cystitis. The latter being th preferred according to the present invention.

Many diseases such as retroperitoneal fibrosis, lupus erythematosus, polyarteritis nodosa, sclerodermia, polymyositis, dermatomyositis, rheumatoid arthritis, anaphylaxis, serum sickness, hemolytic anaemia, allergic agranulocytosis may be succesfully treated according to the present invention with respect to the disease impact on the function of the urinary tract system.

Another important embodiment is the conditions wherein diabetes mellitus is involved.

The primary effect of the treatment according to the present invention is wherein the condition is kidne is recognized as renal failure, nephrotic syndrome or complete or partial urnary tract obstruction, postoperative polyuria.

Most of the condition referred to is associated with reduced renal function indicated by one or more of the following conditions; reduced renal blood flow, reduced glomerular filtration rate, reduced urinary concentrating ability, reduced urinary concentration capacity, reduced or increased urinary electrolyte excretion (such as sodium, potassium, bicarbonate) which may be treated or prevented according to the invention.

Accordingly, the condition may be associated with dysregulation of one or more renal sodium transporters.

Such downregulation of one or more renal sodium transporters may be of at least 50 %, such as at least 75% compared to non-treatment. The sodium transporters may be selected from the group consisting of Na,K-ATPase, NHE-3, NaPi-2, BSC-1, TSC and ENaC's.

Also dysregulation of one or more renal aquaporins may characterize a condition to be treated according to the invention including downregulation of one or more renal aquaporins including aquaporins selected from aquaporins 1 to 12, preferably aquaporins 1 to 4.

The administration according to the use and method of the present invention may be any administration known in th art as may easily be recognise by the skilled person according to the indevidual situation. Accordingly, the administration may be selected from systemic administration; injection into tissue or into a to body cavity including joints; implantation into tissue or into a body cavity; topical application to the skin or to any gastrointestinal surface, or to a mucosal surface including the lining of body cavities.The administration may be selected from parenteral adminstration, including intraperitonal administration, intrathecal administration systemic administration, local administration, topical administration, transmucosal administration and transdermal administration and oral administration.

With respect to an α-MSH equivalent according to the invention, any substance which has an functional effect on the α-MSH receptor such as on the MT1 and/or on the melanocortin receptor is within the scope of the present invention. Many α-MSH equivalents are known in the art including olypeptides having at least 3 amino acids including the following sequence Lys-Pro-Val, such as Gly-Lys-Pro-Val, or the following sequence His-Phe-Arg.

The treatment or prevention as describe above is in a preferred embodiment performed with a combination with a dosage unit of EPO and/or an EPO equivalent. In this respect, the effective dosage of the unit of EPO and/or an EPO equivalent is lower than the dosage in which EPO is generally used for its known indications. The necessary dosage of EPO will generally be a completely non toxic dosage for the individual.

By use of the combination a of an α-MSH or α-MSH equivalent with EPO and/or an EPO equivalent, a synergistic effect may be obtained. The synergism may during a treatment period be is of at least 5% or even higher such as of least 10%, preferebly at least 15% as measured according to a test system in an organ. It is believed that a synergistic effect of at least 20% such as at least 25% may be demonstrated by a treatment according to the invention.

Accordingly, the present invention furhter relates to the use of α-MSH and/or a equivalent of α-MSH for the preparation of a medicament for treatment or prevention of any of the conditions shown herein.

The medicament may be prepared with pharmaceutically acceptabe carriers known in the art or prepared in order to provide a relevant formulation.

Relevant formulations according to the invention includes pharmaceuticals for injection or for systemic administration, wherein the medicament is in a form suitable for injection or systemic administration, e.g. a solution or a suspension. The preparation of a medicament may furher be for implantation, characterized in that the medicament is in incorporated into a coating of a medicotechnical device or is incorporated into the material of the device itself.

The medicament may be for for topical application in the form of a powder, paste, ointment, lotion, gel, cream, emulsion, solution, suspension, spray, aerosol, sponge, strip, plaster, or pad or for oral administration in the form of tablets, sustained release tablets, resoritablets. The mediament may also be a medicament for topical application in the form of a preparation suitable for application on mucosa e.g. a suppository, a tampon, a suspension for irrigation, a tablet or troche, a cream or gel or ointment; or for application on urethral mucosa, a bladder insert or an implant.

The α-MSH and/or α-MSH equivalent is present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament.

Similarily, the method and use also applies for use of an effective dosage EPO and/or of an EPO equivalent and for the combination.

By EPO equivalent according to the present invention is meant any substance which has a functional effect on an EPO receptor.

The EPOand/or EPO equivalent may be present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament. The EPO and/or EPO equivalent is generally uses in dosages completely non-toxic to a human. By use of a combination of a EPO and/or EPO equivalent together with a unit dosage of of α-MSH and/or of an α-MSH equivalent, an effect of the combination may be obtained which is higher than the effect obtained with any of the substances administered alone.

With respect to the combination is possible to obtain an synergistic effect wh re the EPO and/or EPO equivalent and the of α-MSH and/or of an α-MSH equivalent is administered independently of each other. The time span from the release of one of the active ingredients to the organ or tissue in question until the other active ingredient is subjected to the tissue or organ may be several days even 5 days or one week. However, the drugs a preferable administered within at least 48 hours, preferably within 24 hours, such as within 12 hours. However, the practical reasons the active ingredients will normally be substantially co-administered. Possible with a view to the pharmacological and kinetic properties of the individual substances used in the specific treatment.

In a further embodiment the present invention relates to a pharmaceutical composition comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent together with a suitable pharmaceutical carrier. The carrier may be selected according to the specific use as disclosed above. In a furher aspect, the composition may be specificeally adapted for any of the uses and methods disclosed herein.

A further aspect of the invention is a pharmaceutical kit comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent together with a suitable pharmaceutical carrier and optionally a description of the specific use. The kit may comprise the an α-MSH equivalent or α-MSH in any of the forms described herein and the EPO and/or EPO equivalent may be in an identical for or in any other form. Accordingly, the α-MSH may be present in a device for sustained effect wheras the EPO may be present in the kit in a form suitable for injection. The specific kit may accordingly be designed for the individual treatment of prophylactic use.

Example 1 Demonstration of the effect

Introduction

Ureteral obstruction

Bilateral ureteral obstruction (BUO) is associated with reduction in renal functions (Jaenike, J.R., 1972). Characteristically, long-term loss of urinary concentration capacity is a common finding in both children and adults. We and others have shown that BUO and

release of BUO in rats is associated with th onset of a dramatic postobstructive diuresis (POD) and a reduction in urinary concentrating capacity (Sonn nberg, H., 1976). Importantly, we demonstrated that a BUO and release of BUO in rats was associated with a marked reduction in the protein expression of aquaporin-2 (AQP2), AQP3 and AQP1 (Nielsen, S., 1995; Nielsen, S., 1993; Sabolic, I, 1995). Unilateral ureteral obstruction (UUO) is a model of renal injury characterized by progressive tubulointerstitial fibrosis and renal damage, while relatively sparing the glomerulus and not producing hypertension or abnormalities in lipid metabolism (Bohle, A. 1987; Kuncio, G.S., 1991; El Nahas, A.M., 1995; Strutz, F., 1994; Strutz, F., 1995). Irrespective of the underlying cause, many kidney diseases lead to tubulointerstitial inflammation and eventual interstitial fibrosis with permanent loss of renal function (Bohle, A. 1987; Kuncio, G.S., 1991; El Nahas, A.M., 1995; Strutz, F., 1994; Strutz, F., 1995). Most medical investigators agree that new therapeutic strategies should be targeted at developing effective methods for inhibiting renal fibrogenesis. The mechanisms responsible for UUO-induced kidney fibrosis are not well understood. However, prolonged obstruction induces progressive renal fibrosis with dysfunction which cannot be readily restored even with removal of the obstruction. Under these circumstances, pharmacotherapeutic intervention needs to be developed to reverse or halt the progression of the renal dysfunction that occurs as a consequence of the obstruction. Furthermore, the development of progressive interstitial fibrosis represents a final common pathway associated with a variety of kidney disorders that can lead to functional insufficiency (Bohle, A. 1987; Kuncio, G.S., 1991; Strutz, F., 1995). Thus, the search for effective treatment preventing the progression is of great importance not only for elucidating the mechanism of UUO-induced fibrosis, but also for alleviating the renal fibrosis seen under various conditions with chronic renal failure (CRF).

UUO results in changes in renal hemodynamics, infiltration of the kidney by macrophages, and subsequent fibrosis of the tubulointerstitium (Ishibashi, K., 1997). Many of the pathophysiological alterations associated with renal disease are driven by the intercrine, autocrine, paracrine, and endocrine effects of angiotensin II and it has been have demonstrated that angiotensin II production is rapidly stimulated following the onset of ureteral obstruction (Ecelbarger, M. 1994). Angiotensin II, in turn, upregulates the expression of other factors including transforming growth factor- (TGF-) (Kaneto, H., 1993), tumor necrosis factor- (TNF-) (Kaneto, H., 1996), nuclear factor-B (NF-B) (Morrisey, J.J., 1997), adhesion molecules (Morrisey, J.J., 1998; Ricardo, S.D., 1996), and chemoattractants (Diamond, J.R., 1994; Morrisey, J.J., 1998), matrix proteins (Kaneto, H., 1993; Sharma, A.K., 1993), and -smooth muscle actin (-SMA) (Ishidoya, S.,

1995; Nagle, R.B., 1973). The role of TNF- in th pathophysiology of obstructive uropathy, when compared with angiotensin II, is not well understood. In rats, pharmacological maneuvers has been applied to inhibit angiotensin II formation or its biological action through receptor inhibition (Ishidoya, S., 1995; Kaneto, H., 1994; Kaneto, H., 1996; Klahr, S., 1998; Moriyama, T, 1998; Morrissey, J.J., 1997; Morrissey, J.J., 1999). No such pharmacological treatments are available to decipher the biological actions of TNF-. Two different cell surface receptors exist for TNF-, which are designated TNFR1 and TNFR2, that are derived from separate gene products (26). Moreover it has been shown that TNF- contributes, in part, to changes in interstitial volume, myofibroblast differentiation, and NF-B activation in the kidney during ureteral obstruction and are mediated through both the TNFR1 and TNFR2 gene products (mouse study). Thus the angiotensin II and TNF- systems appear to interact with each system, contributing to overall renal fibrosis. Also apoptosis plays a role and it has been shown that the expression of apoptotic and chemokine genes are significantly upregulated in UUO, and bioflavonoids and angiotensin inhibitors are able to attenuate the expression of these genes and thus may be beneficial in renal protection (Jones,E.A., 2000).

There are no realy good treatment of these conditions: 1) The effect of anti-inflammatory treatment with corticosteroids (methylprednisolone) in patient with urinary tract obstruction caused by stones showed that treatment alone did not affect stone passage but combined treatment with the calcium antagonist treatment (nifedipine + methyl prednisolone) facilitated ureteral stone passage (Borghi, 1994). In a few other studies it has been shown that coricosteroid treatment most likely is able to reduce the oedema of the ureteral wall associated with ureteral obstruction (Chye, 1994). 2) The effect of nonsteroidal anti-inflammatory treatment with cyclooxygenase inhibitors such as indomethacin, toradol and sulindic acid, agents which block prostaglandin synthesis, all have shown to have a some beneficial effect on stonepassage and reduce pains associated with ureteral obstruction (Perlmutter, 1993; Frøkiær, 1993). The mechanism involved are speculated to be due to a direct effect on ureteral contraction, reduced oedema of the ureteral wall which in turn may reduce ureteral pressure. The effect to reduce pain by effecting ureteral pressure may also be due to a direct side effect on renal function where GFR and RBF are reduced due to blockade of the effects of vasodilating prostaglandins on renal hemodynamics. Thus a drug with major effect on renal and urinary tract function during obstruction and other kidney disorders associated with fibrosis etc is warranted.

Nephrotic syndrome:

Another common cause of renal failure is nephrotic syndrome, which is caused by glom rular damag and can be a result of treatment with drugs such as adriamycin or purimycin (PAN) aminoglycosides (and other drugs) but the underlying cause of most cases remains unidentified and many patients progress into renal failure (for references see (Apostol et al 1996; Fernandez-Llama, 1998 and 1999)). Nephrotic syndrome is associated with severe proteinuria, systemic edema including acites, hypoproteinemia and hyperlipidemia. It is also associated with decreased urinary concentrating capacity and dilutional ability, and severe sodium and water retention is a cardinal feature in nephrotic syndrome leading to acites, and progression into renal failure. The intrarenal factors leading to the dysregulation of kidney function are not well understood and currently there is no good treatment that prevents the progression of kidney damage. The glomerular changes and possibly also the tubular changes that results in glomerular and tubular dysfunction leading to heavy proteinuria, sodium and water retension and massive peripheral edema have been speculated to be secondary to infiltration with neutrophils, macrophages and monocytes (and induction of many cytokines and adhesion molecules), although this remains less well defined. Also insufficient proliferation and apoptosis in glomerular epithelial cells may be involved in the progression. Thus an inflammatory response is likely to participate during some stages of the development of renal failure (Berens KL 1998; Hori et al; Smoyer et al).

A number of renal transporters and channels have been shown to be dysregulated in association with nephrotic syndrome and this is likely to contribute to the derangement in kidney function. It has been speculated that the decrease in transporter expression in experimental nephritic syndrome is a direct effect of the causing agents (e.g. adriamycin or PAN) on the renal tuble and glomerual epithelial cells (Apostol 1996l; Fernandez-Llama 1998 and 1999). The mechanism by which this agent produces obliteration of the foot processes of glomerular podocytes is not known, but the effect presumably involves impairment of the vesicle trafficking processes involved in maintaining the complex shape of these cells. It appears possible that the decline in ion transporter and water channel expression induced by adriamycin is a consequence of a similar impairment of trafficking in renal tubule cells although this is not established.

Nephrotic syndrome differs markedly from ishemia-induced acute renal failure which is due to reduced or complete arrest in blood supply to the kidney(s). Nephrotic syndrome is often caused (as described above) by drugs (aminoglycosides and other antibiotics), infections, autoimmune disease, connective tissue diseases, cancer and many immune-

mediated forms of glomerulonephritis and is never induced by ischemia. Ischemia is mainly associated with tubular damage whereas nephritic syndrome is mainly a glomerular dis ase with secondary affection of the tubular system. Thus the etiology is completely different.

Cystitis

Interstitial cystitis (van de Merwe, 2000) affects a large number of mature women and the etiology is virtually undefined. For example, patients receiving CYP for the treatment of neoplastic disorders often exhibit side effects of CYP therapy that include: hemorrhagic cystitis, irritative voiding, and gross hematuria. In addition, patients with interstitial cystitis, which is a painful, chronic urinary bladder inflammation syndrome, exhibit urinary frequency, urgency, and suprapubic and pelvic pain. When CYP is injected systemically for the treatment of cancer, the urinary bladder is the organ most affected by the toxic actions of acrolein, a metabolite of CYP excreted in the urine. Histological analysis of the urinary bladder in interstitial cystitis patients shows edema, vasodilation, and proliferation of nerve fibers with chronic infiltration of inflammatory cells such as mast cells. Animal studies have also indicated that CYP treatment in the rat induces cystitis, which is characterized by histological changes in the urinary bladder as well as increased frequency of voiding in awake rats and urinary bladder hyperreflexia in anesthetized rats (Johansson et al 1994)

Melanocortins

Melanocortins are proopiomelanocortin-derived mammalian peptide hormones that include adrenocorticotropic hormone [ACTH (1-39)], alpha- melanocyte-stimulating hormone [alpha-MSH (1-13)], and related amino acid sequence including beta and gamma MSH. Melanocortin peptides have potent antiinflammatory/anticytokine activity (Lipton and Catania 1997). Melanocortins excert at least some of their effect via stimulation of melanocortin receptors. For melanocyte stimulating hormones (MSH) the action is in part ascribed to binding and activation of type 1-5 melanocortin receptors (MC1-MC5).

Melanocortins have a variety of functions including immunomodulation, anti-inflammation, body temperature regulation, pain perception, aldosterone synthesis, blood pressure regulation, heart rate, vascular tone, brain blood flow, nerve growth, placental development, synthesis/release of a variety of hormones such as aldosterone, thyroxin,

prolactin, FSH. ACTH has a major effect on stimulating steroidon ogenesis. Also alpha-MSH induces pigment formation in skin.

Fiiv genes encoding melanocortin receptor subtypes have been identified (MC-receptor type 1-5) (Chhajlani1993, Chhajlani 1992, Gantz1993, Mountjoy1992). The MC receptors belong to the class of G-protein coupled receptors and have seven membrane spanning domains. All receptor subtypes involve increased production of camp to excert their actions. Type 2 receptor (MC2) represent the ACTH receptor whereas the other subtypes are melanocyto stimulating hormone receptors (MSH-receptors).

A series of studies have been performed on the MC receptors in a variety of tissues. Type 1 receptor (MC1), to which alpha-MSH binds with great affinity, is known to be expressed in several tissues and cells such a brain (Xia1995, Rajora1997)) including astrocytes (Wong1997), testis (Vanetti1994), ovary, macrophages (Star1995), neutophils (Catania1996). However MC1 is likely to be expressed in an even wider range of tissues although this remains to be established. The selectivity for the MC=s to bind different melanocortin peptides vary (Schiöth1995-96). MC1 binds with great affinity alpha-MSH and with lower affinity also beta-MSH, gamma-MSH and ACTH. MC2 has been reported only to bind ACTH but non of the MSH peptides. The highest affinity for the ligands of the other receptors including gamma-MSH (MC3-receptor), beta-MSH (MC4-receptor). In contrast MC5 binds with much lower affinity the MSH peptides but with the same pattern as MC1 (i.e. highest affinity for alpha-MSH).

It is important to emphasize that a number of actions of MSH peptides, especially alpha-MSH, are not fully established with respect to which receptors are involved. The prominent anti-inflammatory action of alpha-MSH has been speculated to involve a variety of processes including interference with NO production, endothelin-1 action, interleucin 10 formation, which again is linked to MC1 receptors expressed in macrophages, monocytes (Star1995; Bhardwaj1996).

Alpha-MSH has also been shown to be important in a variety of infllamtory processes (Lipton and Catania 1997): 1) inhibit chemotactiv migration of neutrophils (Catania1996). 2) alpha-MSH including analogs inhibit the release of cytokine (IL-1, TNF-alpha) in response to LPS treatment (Goninard1996). 3) Inhibit TNF-alpha in response to bacterial endotoxin (Wong1997). 4) ICV or IP administration of alpha-MSH inhibit central TNF-alpha production by locally administered LPS. 5) alpha MSH has been shown to reduce the inflammation in experimental inflammatory bowel disease (Rajora1997), ishemia-

induced acute renal failure (Star1995,Kwon1999). 6) Alpha-MSH also have some protective effect by inhibiting the induction and elicitation of contact hypersensitivity and induces hapten tolerance, and it is speculated that alpha MSH may mediate important negative regulation of cutaneous inflammation and hyper-prolefirative skin diseases (Luger1997). To this end alpha-MSH causes increased IL-8 release from derman microvasculature endothelial cells (Hartmeyer1997).

Erythropoetin (EPO) The cellular adaptation to hypoxia involves many changes in gene expression, such as those of erythropoietin (Epo), vascular endothelial growth factor (VEGF), glycolytic enzymes, and tyrosine hydroxylase. Several reports have demonstrated that both oxygen sensing and chemical signaling occur via a common pathway that leads to the activation of hypoxia-induc-ible factor-1 (HIF-1) (Semenza and Wang, 1992; Wang and Semenza, 1993a,b; Wenger and Gassmann, 1997), a transcription factor which is induced over a physiologically relevant range of oxygen tensions (Jiang et al., 1988; Jelkmann, 1992; Ratcliffe et al., 1997; Bunn et al., 1998). Epo is a 34-kDa glycoprotein hormone which has been characterized as the principal regulator of erythropoiesis and was thought to be exclusively produced in fetal liver and adult kidney in response to hypoxia (Zanjani et al., 1977; Fandrey and Bunn, 1993; Kramer et al., 1997). The molecular biology of the oxygen sensing mechanism underlying the transcriptional activity of Epo has been intensively investigated in HepG2 and Hep3B human hepatoma cell lines. In addition to transcriptional activation by HIF-1, mRNA stabilization has been found to account for an accumulation of Epo mRNA (Schuster et al., 1989; Goldberg et al., 1991; Fandrey and Bunn, 1993; Bunn et al., 1998). Agents such as cobalt chloride (CoCl2) and desferrioxamine (DFX) are able to mimic the hypoxia-induced Epo tran- scription (Goldberg et al., 1987, 1988; Wang and Se-menza, 1993c; Ehleben et al., 1997; Porwol et al., 1998).

Indirect evidence has been provided to indicate that redox-mediated processes are likely to be involved in the induction of the EPO gene (Fandrey et al., 1994, 1997; GoØ rlach et al., 1994; Wang et al., 1995; Salceda and Caro, 1997; Canbolat et al., 1998). Thus, iron and reactive oxygen species might play a critical role in the oxygen sensing mechanisms involved in the regulation of the expression of the EPO gene. Recent reports suggest that, along with its role in erythropoiesis, EPO might be of biological significance in the central nervous system. In vivo, Epo mRNA is expressed in both rodent and primate brain tissues and its expression is increased following hypoxia (Tan et al., 1992; Digicaylioglu et al., 1995; Marti et al., 1996). Taken together, several findings imply that Epo acts on neurons

in a paracrine way. This notion has been support d by th in vitro and in vivo europrotective effects of Epo (Morishita et al., 1997; Sadamoto et al., 1998; Sakanaka et al., 1998; Bernaudin et al., 1999). Several groups (Sadamoto et al., 1998; Sakanaka et al., 1998; Bernaudin et al., 1999) have shown that the direct administration of EPO to the central nervous system of mice, rats, and gerbils significantly reduces neuronal death and prevents learning disability asso-ciated with cerebral ischemia.

Methods

Experimental animals

Studies were performed on adult male Munich Wistar rats (Møllegard Breeding centre Ltd., Eiby, Denmark).The rats were maintained on a standard rodent diet (Altromin, Lage, Germany) with free access to water. During the entire experiment, rats were kept in individual metabolic cages, with a 12:12h artificial light/dark cycle, a temperature of 21, plus/minus 2C°, and a humidity of 5 5%. Rats were allowed to acclimatize to the cages for 3 days prior to surgery.

Induction of bilateral ureteral obstruction

Rats were anesthetized with halothane and placed on a heating Board under an operating microscope, Through a midline abdominal incision both ureters were exposed and occluded by placing a 5-mm piece of bisected polyethylene tubing (PE-50) around the midportion of each ureter. The ureter was then occluded by tightening the tubing with a 5-0 silk ligature. 24 hours later, the obstructed ureters were decompressed by removal of the ligature and the PE-50 tubing. Done in this manner, both ureters could be completely occluded for 24 h and without evidence of functional impairment of ureteral function.

*Induction of unilateral ureteral obstruction*Rats were anaesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and placed on a heating board under an operating microscope. Through a midline abdominal incision the left ureter was exposed and a 5 mm piece of bisected polyethylene tubing (PE-50) was placed around the midportion of the ureter. The ureter was then occluded by tightening the tubing with a 5-0 Silk ligature. Twenty-four hours later, the obstructed ureters were decompressed by removal of the ligature and the PE tubing. Using this technique the ureter could be completely occluded for 24 hours without evidence of subsequent functional impairment of ureteral function.

Induction of purimycin and adriamycin-induced nephrotic syndrome in rats

Nephrotic syndrome was induced by a single i.p. or s.c. injection of adriamycin or purimycin (at various doses) and th rats were followed for 7 days B 21 days. Development of severe nephrotic syndrome was established my monitoring the total body weight, urinary protein excretion (proteinuria) and at time of ending the experiment the volume of fluid in the abdominal cavity was determined (rats develop severe water retention and acites). The effect of alfa-MSH or epoetin or alfa-MSH combined with epoetin was determined by treatment with the compounds for the initial 3 days after adrimycin or purimycin administration.

Experimental protocols

The following protocols were performed:

Protocol 1: This protocol included 1) Rats with BUO for 24 hours (n=20) and 2) sham-operated rats (n=10). The BUO animals were divided into two groups: alfa-MSH nontreated (n=10) and alfa-MSH treated (n=10). alfa-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50μg, i.v.) was given at the onset of BUO and 12 hours later.

Protocol 2: This protocol included 1) Rats with BUO for 24 hours and followed by release for 5 hours(n=10) and 2) Sham operated rats (n=5). The rats with BUO-R were divided into two groups: alfa-MSH nontreated (n=5) and alfa-MSH treated (n=5). Alfa-MSH (50±g, i.v.) was given at the onset of BUO, 12 hours later and at the onset of release.

Protocol 3: This protocol included 1) Rats with BUO for 24 hours and followed by release for 48 hours. The rats were divided into two groups: alfa-MSH nontreated (n=10) and alfa-MSH treated (n=13). Alfa-MSH (50μg) was given with micro-osmotic pump via jugular vein at the onset of BUO. 2) Sham operated rats (n=8) treated with vehicle with micro-osmotic pump.

Protocol 4: This protocol included rats which had a detailed examination of renal function before onset and after release of BUO. 1) Rats with BUO for 24 hours followed by release for 48 hours (n=10) and 2) sham-operated rats (n=5). The BUO animals were divided into two groups: alfa-MSH nontreated (n=5) and alfa-MSH treated (n=5). Alfa-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50μg, i.v.) was given at the onset of UUO and 12 hours later.

Protocol 5: This protocol included 1) Rats with UUO for 24 hours (n=11) and 2) sham-operated rats (n=5). The UUO animals were divided into two groups: alfa-MSH nontreated (n=5) and alfa-MSH treated (n=6). -MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50μg, i.v.) was given at the onset of UUO and 12 hours later.

Protocol 6-10: These protocols included examinations similar to those described in protocols 1-5, where rats were treated with [Nle^4,D-Phe^7]- α-MSH instead of α-MSH.

Protocol 11: This protocol included 1) Rats with UUO and BUO for 24 hours and 2) sham-operated rats . The UUO and BUO animals were divided into two groups: Epoitin treated (100 U/kg/day i.p.) and non-treated rats.

Protocol 12: This protocol included 1) Rats with UUO and BUO for 24 hours and 2) sham-operated rats . The UUO and BUO animals were divided into two groups:One group was treated with a combination of epoitin (100 U/kg/day i.p.) and α-MSH (50μg, i.v.) was given at the onset of UUO/BUO and 12 hours later.

Protocol 13. Rats were treated with adriamycin (7.5 mg/kg i.p.; n=12) and followed for 21 days, respectively. Shams received saline i.p. (n=6). Half of the adriamycin treated animals received alfa-MSH in osmotic minnipumps (50 ug/day) during the entire experiments. The other half received vehicle (saline) in osmotic minipumps. In another series of experiments alfa-MSH treatment was administered (at the time of adriamycin injection and 6, 24 and 48 hours after adriamycin injection).

Protocol 14. Rats were treated with purimycin (100 mg/kg i.p., n=6) and followed for 11days, respectively.Half recieved alfa-MSH treatment (at the time of PAN injection and 6, 24 and 48 hours after PAN injection).

Protocol 15. Rats were treated with adriamycin (7.5 mg/kg i.p.; n=8) and followed for 21 days, respectively. Shams received saline i.p. (n=6). Half of the adriamycin treated animals received epoetin (100 units/kg/day) during the entire experiments. The other half received vehicle (saline).

Protocol 16. Rats were treated with purimycin aminoglycoside (100 mg/kg i.p., n=6) and followed for 11 days, respectively.Half recieved epoetin (100 units/kg/day).

Protocol 17. Rats were treated with purimycin (100 mg/kg i.p., n=6) and followed for 11days, respectively.Half recieved alfa-MSH treatment (at the time of PAN injection and 6, 24 and 48 hours after PAN injection) and in addition they received epoetin (100 units/kg/day).

Protocol 18. Temporary ligation of intestinal blood supply for 15-30 min followed by reperfusion. A variety of parameters were determined including mean arterial blood pressure, glomerular filtration rate etc.

Protocol 19. Chemical cystitis was induced in Wistar rats of either sex (200-250 g) by CYP, which is metabolized to acrolein, an irritant eliminated in the urine. CYP (Sigma Chemical, St. Louis, MO; 75 mg/kg ip) was administered every 3rd day for 2 wk to elicit chronic irritation. All injections of CYP were performed under halothane (2%) anesthesia.

Animals were either non-treated or treated with EPO or alpha-MSH or with a combination of EPO and alpha-MSH using the same protocol as used for PAN nephrosis.

Operative procedures:

Catheterisation of the Jugular Vein

In protocols 2,3,4,7,8 and 9 the jugular vein was exposed at least 1 cm. The tip of the catheter was inserted into the vein and pushed forwards towards the heart (about 2.5cm) filled with a heparin-saline solution ("heparin lock"). The catheter was then tied into the blood vessel with the ligature. With a small incision made in the dorsal nape of the neck the catheter was passed subcutaneously from the site of the entry of the catheter of the jugular vein to the dorsal incision down the side of the neck to emerge anterior. And a micro-osmotic pump was connected with the end of catheter.

Permanent bladder catherization

In protocols 4 and 9 catheters were permanently placed in the bladder for urine collection. One week before the experiment, the animals were anesthetized with halothane/N_2O. Using aseptic surgical techniques, sterile Tygon™ catheters (Norton Performance Plastics, Arkon, OH) were advanced into the abdominal aorta and the inferior vena cava via the femoral vessels. A sterile chronic suprapubic catheter was implanted into the bladder. All catheters were produced and fixed, with minor modifications, as described previously (7). After instrumentation, the rats were infused with saline subcutaneously (5 ml) and given a long-acting analgesic, Buprenorphinum (Temgesic™ ; Reckitt & Colman, Hull, United Kingdom), subcutaneously and housed individually. After a recovery period of 5 to 6 d, the rats were acclimatized to restriction by daily training sessions in restraining cages. The duration of each daily session was gradually increased from 1 to 3 h a day.

Primary Antibodies

For semiquantitative immunoblotting, previously characterized mouse monoclonal and affinity purified rabbit polyclonal antibodies were used:

1) AQP2 (LL127 1:6000): An affinity purified polyclonal antibody to AQP2 (5)

2) AQP1 (LL266 1:3000): An affinity purified polyclonal antibody to AQP1(40)

3) AQP3 (LL178 1:400): An affinity purified polyclonal antibody to AQP3 (6;42)

4) AQP4 (LL182AP): An affinity purified polyclonal antibody to AQP4 has previously been characterized (li55).

4) NHE-3 (LL546AP): An affinity purified polyclonal antibody to NHE-3 has previ usly been characterized (li--13,26,31).

5) NaPi-2 (LL696AP): An affinity purified polyclonal antibody to type II Na-Pi cotransporter (NaPi-2) which was raised against the final 24 amino acids of COOH-terminal sequence has previously been characterized (li-5).

6) Na,K-ATPase: A monoclonal antibody against the alpha-1 subunit of Na,K-ATPase has previously been characterized (li-24).

7) BSC-1 (LL320AP): An affinity purified polyclonal antibody to the apical Na-K-2Cl cotransporter of the thick ascending limb has previously been characterized (li-12,26,47).

8) TSC (LL573AP): An affinity purified polyclonal antibody to the apical thiazide-sensitive Na-Cl cotransporter of the distal convoluted tubule has previously been characterized (li-28).

Clearance studies

Weight , water intake , food intake and urine output were observed during the rats were maintained in the metabolic cages. Urine was collected over 24-h periods throughout the study. Urine volume, osmolality, creatinine, sodium and potassium concentration were measured. Plasma was collected from abdominal aorta at the time of sacrifice for measurement of sodium and potassium concentration, creatinine, and osmolality.

In protocols 4 and 9 detailed examinations of renal function was performed: The experiments were carried out between 8 a.m. and 1 p.m. The rats were transferred to a restraining cage and connected to infusion pumps via the vein catheter and to a BP transducer via the arterial catheter. Urine was collected in three periods of 20 min preceded by an equilibration period of 105 min. Throughout the experiment, a half isotone saline (77 mM NaCl) was infused at a rate of 70 µl/min to maintain a minimum urine flow necessary for accuracy of the bladder emptying. ^{14}C-tetraethylammonium bromide (0.83 µCi/ml ; New England Nuclear, Boston, MA), together with ^{3}H-inulin (2.5 µCi/ml ; Amersham, Rainham, United Kindgom) and LiCl (13 mmol/L), were infused together with the saline as markers of effective renal plasma flow (ERPF), GFR, and tubular fluid delivery from proximal tubules (V_{prox}), respectively. A bolus of markers four times the continuous infusion velocity was given in the first 15 min. Blood samples (200 µl) were drawn from the arterial catheter after 105 and 165 min. Blood substitution with donor blood was given after each blood sample. Mean arterial BP was recorded continuously using a Uniflow™ transducer (Baxter, Irvine, CA) connected to a preamplifier and PC registration.

Clearance experiments were carried out in BUO and SHAM rats 7 days prior to obstruction and 48 hours after release of BUO.

AnalysisUrine volume was determined by gravimetric means. Li^+ concentration was determined in plasma and urine by flame emission photometry and atomic absorption spectrophotometry, respectively. ^{14}C-tetraethylammonium (TEA) and ^{3}H-inulin in plasma and urine were determined by dual label liquid scintillation counting (Wallac™ model 1409 ; Helsinki, Finland). Sample (15 l) and 285 µl of water weremixed with 2.5 ml of scintillation liquid (Ultima Gold™ ; Packard Instruments, Meriden, CT). Correction of dpm was performed by automatic efficiency control.

CalculationsRenal clearances (*C*) were calculated by the standard formula :

$C = U \times V/P$

where *U* is urine concentration, *V* is urine flow rate, and *P* is plasma concentration.

In previous studies, the renal extraction fraction of TEA has been shown to approximate 90%, and the validity of TEA as an estimate of ERPF has been documented (Ref). By use of C_{TEA}, C_{IN}, and C_{LI}, the following parameters were calculated :

ERPF = Effective renal plasma flow (CTEA)

GFR = Glomerular filtration rate (Cin)

Membrane fractionation for immunoblotting

Inner medulla and whole kidneys were homogenized (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing 8.5 M leupeptin, 1 mM phenylmethyl sulfonylfluoride) using an ultra-turrax T8 homogenizer (IKA Labortechnik, Germany), at 5 strokes for 20 seconds (inner medulla) or at 5.5 strokes for 30 seconds (whole kidney) and the homogenate was centrifuged in an Eppendorf centrifuge at 4000 g for 15 minutes at 4°C to remove whole cells, nuclei and mitochondria. Gel samples (Laemmli sample buffer containing 2% SDS) were made of this pellet.

Electrophoresis and immunoblotting

Samples of membrane fractions from inner medulla and total kidney were run 12% polyacrylamide minigels (Bio-Rad Mini Protean II) for AQP1, AQP2 and AQP3. For each gel, identical gel was run in parallel and subjected to Coomassie staining to assure identical loading (40).Then gels were subjected to immunoblotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and

incubated overnight at 4°C with affinity-purified primary antibodies (s e above). Th labeling was visualized with horseradish peroxidase (HRP)-conjugated secondary antibodies (P448, DAKO, Glostrup Denmark, diluted as 1:3000) using an enhanced chemiluminescence system (ECL, Amersham International, UK).

Quantitation of total kidney levels of AQPs.

ECL films with bands within the linear range were scanned using an AGFA scanner (ARCUS II) and Corel Photopaint Software to control the scanner. For AQP1 and AQP2, both the 29-kDa and the 35- to 50-kDa bands (corresponding to nonglycosylated and the glycosylated species) were scanned. For AQP3, both the 27-kDa and the 33- to 40-kDa bands (corresponding to nonglycosylated and the glycosylated species) were scanned. The labeling density was determined of blots where samples of kidneys from alfa-MSH treated and nontreated groups were run together with samples from control kidneys. The labeling density was corrected by densitometry of coomassie stained gels. (i.e. to control for minor difference in loading)

Quantitation of urinary protein excretion

To determine the levels of proteinuria in experimental nephrotic syndrome urine was examined daily using Albym (Boehringer-Mannheim).

Statistical analyses

Values were presented in the text as means ± standard errors. Comparisons between groups were made by unpaired t-test. *P* values < 0.05 were considered significant.

Results

BUO for 24 hours , 5 and 48 hours after release of BUO are associated with reduced AQP1 , AQP2 and AQP3

As previously studied, immunoblotting revealed that 24 h of BUO and 48 hours after release of BUO were associated with a significant downregulation of AQP-2 expression compared with sham-operated controls (Table 1) (9). Semiquantitative immunoblotting from the inner medulla of rats with 24h BUO and BUO-R for, 5 and 48 hours revealed that AQP3 expression was persistently downregulated (Table 1). Both AQP3 bands (the 27-kDa and 33- to 40-kDa bands) were decreased proportionately. Densitometric

analysis revealed a significant decrease in AQP3 expression in rats with 24 h BUO to 19±4% of sham levels (100±7%, p<0.05)(Table 1). Furthermore, AQP3 prot in levels were marked decreased at 5 hours after release to15±5% of sham levels (100±9%, p<0.05) and at 48 hours after release to 10±5% of sham level (100±1%, p<0.05).

Semiquantitative immunoblotting using membrane fractions prepared from the whole kidney of rats with 24h BUO and BUO-R for 5 and 48 hours revealed that AQP1 expression was persistently downregulated (Table 1). Both AQP1 bands (the 29-kDa and 35- to 50-kDa bands) were decreased proportionately. Densitometric analysis revealed a significant decrease in AQP1 expression in rats with 24 h BUO to 53 7% of sham levels (100 9% p<0.05). Furthermore, AQP1 protein levels remained markedly decreased at 5 hours after release of BUO to 7 ±% of sham levels (100±22%, p<0.05) and at 48 hours after release to 30±5% of sham level (100±10%, p<0.05)(Table 1).

BUO for 24 hours , 5 and 48 hours after release of BUO are associated with downregulation of Na,K-ATPase

Semiquantitative immunoblotting using membrane fraction prepared from whole kidney of rats with 24 h BUO, and BUO followed by 5 h and 48 h after release of BUO showed a persistant downregulation of Na,K-ATPase to 35 - 50% of control levels (Table 1)

alfa-MSH partially prevents AQP2 and AQP3 downregulation in response to 24 h of BUO

Semiquantitative immunoblotting using membrane fractions prepared from the inner medulla of rats with 24 h BUO and sham operated control rats revealed that alfa-MSh treatment significantly increased AQP2 expression were compared with nontreated rats (38±5% vs. 13±4%, p<0.05). At the same time, immunoblotting also showed that expression of AQP3 in rats with 24 h BUO was significantly upregulated in response to alfa-MSH treatment compared with nontreated rats (44±3% vs. 19±4%, p<0.05.

Plasma concentrations data (Table 2) showed that in -MSH treated rats with 24 h BUO plasma sodium levels had a marked increase compared with 24 h BUO rats without -MSH treatment from 135 2 mmol/L to 139 0.6mmol/L. There was no difference between -MSH treated rats andsham operated rats. Otherwise, others plasma data were no change between -MSH treated and nontreated rats.

alfa-MSH prevents AQP3 and AQP1 downregulation in response to 24 h BUO followed by release for 5 hours

Semiquantitative immunoblotting using membrane fractions prepared from the inner medulla of 24 h BUO rats followed by release for 5 hours and sham operated rats revealed that with -MSH treatment AQP3 expression was significantly increased compared with nontreated rats with 24 h BUO followed by release for 5 hours form 14 5% to 34 4% of sham level(100 14%).

Semiquantitative immunoblotting using membrane fractions prepared from whole kidney of rats with 24 h BUO followed by release for 5 hours and sham operated control rats revealed that with -MSH treatment significantly increased AQP1 expression levels compared with nontreated BUO-R rats (81±21% vs. 7±2%, $p<0.05$) (Fig 3 A and B). Furthermore, immunoblotting prepared from outer medulla and cortex also showed the same result that in -MSH treated rats AQP1 expression was a marked increase compared with nontreated rats from 35±2% to 62±9% of sham level.

alfa-MSH prevents AQP1 downregulation in response to 24 h BUO followed by release for 48 hours

In rats with 24 h BUO and 5 hours after release of BUO , alfa-MSH was given every 12 hours. In rats with 48 hours after release of BUO, alfa-MSH was given with micro-osmotic pump continuously. As shown in Figure 3E and 3F, semiquantitative immunoblotting using membrane fractions prepared from the whole kidney of 24 h BUO rats followed by release for 48 hours and sham operated rats revealed that with -MSh treatment the levels of AQP1 expression were significantly increased compared with nontreated rats with 48 hours after release of BUO form 24±5% to 58±6% of sham level(100 10%). Furthermore, immunoblotting prepared from outer medulla and cortex also showed the same result that in alfa-MSH treated rats AQP1 expression was a marked increase compared with nontreated rats to 73±8% of sham level.

alfa-MSH completely normalize expression of Na,K-ATPase during BUO and 5 and 48 hours after release of BUO

Semiquantitative immunoblotting using membrane fractions prepared from whole kidney of rats with 24 h BUO showed that Na,K-ATPase levels were completely normalized (Fig 1 A and B). Also, 24 h BUO followed by 5 h of release and sham operated control rats revealed that -MSH completelynormalized Na-K-ATPase levels (102±14%)(Fig. 2, A

and B). alfa-MSH treatment also completely normalized Na-K-ATPase levels 48 hours after release of BUO (114±10%)(Fig. 4, A and B).

Treatment with alfa-MSH prevents the increase in plasma creatinine and restores GFR and RPF in response to 24 hours of BUO followed by 48 hours of release

Plasma creatinine is an important marker of glomerular filtration rate. In rats with BUO for 24 h followed by 48 of release and sham operated control rats -MSH treatment significantly prevented the dramatic increase in plasma creatinine (indicating severe renal insufficiency). Plasma creatinine levels were significantly reduced in -MSH treated rats (79 34 mol/ vs.160±34 µmol/l, $p<0.05$, Fig. 5). In addition, plasma urea which is another important marker of renal function almost normalized in rats treated with -MSH (13 2mmol/l vs. 35 9mmol/l, $p<0.05$). Also GFR and ERPF were completely normalized (Fig 6 A and B).

Preventive effect of -MSH, or Epoetin treatment on adriamycin and purimycin-induced nephrotic syndrome in rats.

Treatment with -MSH for 3-4 days after i.p injection of adriamycin (protocol 14) significantly prevented the acites production by 89 ± 7% indicating a marked preventive effect of alfa-MSH on experimentally induced nephrotic syndrome. A similar dramatic effect was also observed in low dose purimycin treatment (80±8% reduction, n=6) and a marked reduction in proteinuria (Fig 8). A less potent effect was seen in very servere purimycin (high dose) induced nephrotic syndrome. EPO treatment once every 24 hours also prevented the acites formation in adriamycin-induced nephrotic syndrome and co-treatment of epoetin and alfa-MSH produced an even greater effect.

Preventive effect of -MSH, or Epoetin treatment or combined treatment on intestinal ischemia associated disease in rats.

Treatment with alfa-MSH significantly prevented death in response to temporal intestinal ischemia (Fig 9).

Mechanisms of the preventive effect of alfa-MSH, or Epoetin treatment or combined treatment.

As decribed above (and in Lipton review) alpha-MSH is likely to exert its effect via MC-1 receptors expressed in inflammatory cells (including neutrophils) or directly in the epithelium (not known). The effects of epo outside its effect in stimulating erythropoesis is

virtually undefined but may include leads activation of hypoxia-induc-ible factor-1 (HIF-1) (Semenza and Wang, 1992; Wang and Semenza, 1993a,b; Wenger and Gassmann, 1997), a transcription factor which is induced over a physiologically relevant range of oxygen tensions (Jiang et al., 1988; Jelkmann, 1992; Ratcliffe et al., 1997; Bunn et al., 1998). EPO receptors have been found in multiple tissues including kidney and it is speculated that EPO acts via this receptor although binding to other receptors cannot be excluded.

Curative effect of alfa-MSH, or Epoetin treatment or combined treatment on interstitial cystitis associated disease in rats.

Models of interstitial cystitis in rats pigs revealed that single alpha-MSH or EPO treatment or combined treatment with EPO plus alpha-MSH markedly reduced bladder hyperreactivity, decreased bladder mast cell infiltration and other inflammatory parameters.

Legends

Fig 1. Panel A shows an immunoblot from whole kidney samples reacted with an alfa-1-isoform-specific monoclonal antibody to Na-K-ATPase and revealed an approximately 96kDaband. Panel B shows densitometric analysis. In response to 24h BUO Na-K-ATPase expression was decreased from 100 8% in sham operated controls to 40±10% in nontreated animals (* P<0.05). With ala-MSH treatment the level of Na-K-ATPase expression was greatly increased to 98±18% compared with nontreated 24 h BUO rats (# p<0.05). There was no difference between alfa-MSH treated and sham operated animals, thus alfa-MSH treatment completely prevented the downregulation of Na-K-ATPase in response to BUO.

Fig 2. Panel A shows an immunoblot from whole kidney samples reacted with a alfa-1-isoform -specific monoclonal antibody to Na-K-ATPase and revealed an approximately 96kDa band. Panel B shows densitometric analysis. In response to 24h BUO Na-K-ATPase expression was decreased from 100±15% in sham operated controls to 50±11% in nontreated animals (* P<0.05). With alfa-MSH treatment the level of Na-K-ATPase expression was greatly increased to 102±14% compared with nontreated BUO rats (# p<0.05). There was no difference between alfa -MSH treated and sham operated animals. Thus alfa-MSH treatment completely prevented the downregulation of Na-K-ATPase in response to 24 hours of BUO

Fig 3. Panel A shows an immunoblot from whole kidney reacted with affinity-purified anti-aquaporin-1 (anti-AQP1) which revealed 29 kDa and 35-50 kDa AQP1 bands, representing non-glycosylated and glycosylated forms of AQP1. Panel B shows densitometric analysis. In response to 24 hours of BUO and 5 hours release densitometric analysis of all samples from nontreated, alfa-MSH treated rats with 24-hour BUO and sham-operated controls revealed that AQP1 expression decreased from 100±22% in sham operated controls to 7±2% in BUO rats without alfa-MSH treatment,* P<0.05. alfa-MSH treatment significantly increased the level of AQP1 expression to 81±21% compared with nontreated rats with BUO, # P<0.05. Thus alfa-MSH treatment prevented the dramatic decrease in AQP1 expression in response to 24 hours of BUO and 5 hours of release.

Fig 4. Panel A shows an immunoblot from kidney cortex and outer medulla samples reacted with alfa-1-isoform-specific monoclonal antibody to Na-K-ATPase and revealed an approximately 96kDa band. Panel B shows densitometric analysis. In response to 24h BUO Na-K-ATPase expression was decreased from 100±4% in sham operated controls to 62±3% in nontreated animals (* P<0.01). With alfa-MSH treatment the level of Na-K-ATPase expression was greatly increased to 118 9% compared withnontreated BUO rats (# p<0.01) and also significantly increased compared with sham operated animals (* P<0.01). There was no difference between alfa-MSH treated and sham operated animals. Thus alfa-MSH treatment completely prevented the downregulation of Na-K-ATPase in response to 24 hours of BUO followed by 48 hours of release.

Fig 5. Plasma creatinine levels were increased to 341±8 μmol/l in animals which were subjected to 24 h BUO and released for 48 h. Plasma creatinine levels were significantly reduced in alfa-MSH treated rats (79±34 μmol/l vs.160±34 μmol/l, p<0.05).

Fig 6. Panel A: Glomerular filtration rate (GFR) did not differ among the three groups at baseline level. alfa-MSH treatment completely prevented the reduction in GFR (BUO+MSH: 705 ± 85 μl/min/100g vs. SHAM: 840 ± 105 μl/min/100g) 48 hours after release of BUO. Panel B: Effective renal plasma flow (ERPF) did not differ among the three groups at baseline level. alfa-MSH treatment completely prevented the reduction in ERPF (BUO+MSH: 2598 ± 129 μl/min/100g vs. SHAM: 2633 ± 457 μl/min/100g) 48 hours after release of BUO.

Fig 7. Protocols for the experimental models of aminoglycosid treatment and the effect of alfa-MSH analogs or epoetin treatment (or treatment with epoetin and alfa-MSH analogs in combination).

Fig 8. Determination of proteinuria levels in alfa-MSH or untreated PAN rats compared to SHAM rats at day 5 after PAN injection. A marked reduction is seen in proteinuria demonstrating a dramatic effect of alfa-MSH in preventing to a marked extent the severity in the developement of nephrotic syndrome

Fig 9. Dramatic prevention of disease associated with temporal intestinal ischemia in response to treatment either with alpha-MSH or epoetin or combined treatment with epoetin and alpha-MSH. Single treatment with epoetin or alpha-MSH had a significant effect but the combination theraphy dramatically improves the prevention of organ function or death.

TABLES

Table 1 Expression of AQP1, AQP2 and AQP3 in BUO , release of BUO and sham operated rats

	24hBUO		24hBUO-5hRelease		24hBUO-48hRelease	
	Nontreated	SHAM	Nontreated	SHAM	Nontreated	SHAM
AQP1	0.48±0.07*	1±0.09	0.07±0.02*	1±0.22	0.24±0.05*	1±0.10
AQP2	0.13±0.04*	1±0.07	0.32±0.11*	1±0.04	0.13±0.05*	1±0.01
AQP3	0.19±0.04*	1±0.07	0.14±0.05*	1±0.14	0.10±0.05*	1±0.01
n	10	10	5	5	5	4

Values are means ± SE.
*P<0.05 compared with sham-operated control rats

Table 2. Plasma concentrations data for different groups on last day in each protocol

	24hBUO			24hBUO-5hRelease			24hBUO-48hRelease		
	Nontreated	MSH	SHAM	Nontreated	MSH	SHAM	Nontreated	MSH	SHAM
P-Osm, mOsm/kgH2O	332±4*	345±5*	302±1	336±2*	344±3*	301±2	327±7*	311±2*#	299±2
P-Na, mmol/l	135±2*	139±0.6#	139±0.5	140±0.2	141±1	139±1	137±2	140±1*#	138±0.5
P-K, mmol/l	6.3±0.3*	6.1±0.2*	3.9±0.1	4.9±0.1*	5.6±0.2*#	4.2±0.1	5.2±0.4*	4.6±0.1*	4±0.1
P-Creat., µmol/l	377±10*	366±11*	31.1±0.8	203±17*	234±17*	32.8±1.5	160±34*	79.5±8*#	30.4±0.7
P-Urea, mmol/l	43±2*	44±2*	4.8±0.3	37±1*	40±1*	4.3±0.2	35±9*	13±2.2*#	4.3±0.2
n	10	10	10	5	5	5	10	13	8

Values are means ± SE. BUO, bilateral ureteral obstruction; P-osm, plasma osmolality; P-Na, plasma sodium; P-K, plasma potassium; P-creatinine, plasma creatinine; P-urea, plasma urea.
*P<0.05 compared with sham-operated control rats
#P<0.05 compared to nontreated rats with 48 hours after release of BUO

Table 3. Rat weights, food intakes, water intakes and urine outputs (24hBUO-48hRelease)

	Weight g	Food intake µg·min-1·kg-1	Water intake µl·min-1·kg-1	Urine output µl·min-1·kg-1
Day -3				
Nontreated	255±7	58±1	95±6	42±3
MSH	254±4	56±3	91±4	45±3
SHAM	251±7	48±4	74±9	36±5
Day -2				
Nontreated	248±7	61±1	95±5	42±4
MSH	250±4	58±1	89±4	43±3
SHAM	249±6	61±3	94±6	42±5
Day -1				
Nontreated	252±7	59±2	88±4	42±3
MSH	256±5	60±1	88±3	42±3
SHAM	253±6	60±2	85±5	40±4
Day 0				
Nontreated	251±8	3±1*	36±6	0
MSH	254±5	6±2*#	38±4*	0
SHAM	247±6	29±3	51±47	26±3
Day 1				
Nontreated	216±5*	3±1*	84±107	122±10*
MSH	224±4*	4±1*	89±8	113±6*
SHAM	248±6	44±2	71±3	30±3
Day 2				
Nontreated	214±6*	17±5*	148±9*	105±10*
MSH	229±4*	28±3*#	165±10*	102±9*
SHAM	251±6	51±2	85±3	34±3

Values are means ± SE. Rat weight, food intake, water intake, urine output are from rats 48 hours after release of BUO without α-MSH treatment(n=10) or with α-MSH treatment(n=13) and time-matched sham-operated control rats(n=8)..
*P<0.05 compared with sham-operated control rats
#P<0.05 compared to nontreated rats with 48 hours after release of BUO

Table 4. Renal functional data (24hBUO-48hRelease)

	U-osm mOsmol/kgH_2O	U-Na mmol/l	U-K mmol/l	U-creatinine µmol/l	U-urea mmol/l
Day -1					
Nontreated	1534±244	201±30	280±43	5.8±1.0	619±104
MSH	1552±114	219±29	299±25	6.1±0.7	668±51
SHAM	1821±218	233±20	335±33	7.3±0.8	790±113
Day 0					
Nontreated	0	0	0	0	0
MSH	0	0	0	0	0
SHAM	1679±348	142±26	294±36	9.0±1.2	866±126
Day 1					
Nontreated	530±65*	80±17*	66±11*	1.8±0.2*	178±13*
MSH	533±19*	74±8*	72±4*	2.6±0.2*#	208±17*
SHAM	2132±257	252±24	368±30	10.8±2.0	1015±157
Day 2					
Nontreated	559±38*	42±9*	66±5*	2.2±0.2*	276±8*
MSH	592±68*	32±3*	62±5*	2.5±0.2*	338±48*
SHAM	2262±232	277±37	372±33	10.0±1.5	1085±128

Values are means ± SE. BUO, bilateral ureteral obstruction; U-osm, urineosmolality; U-Na, urine sodium; U-K, urine potassium; U-creatinine, urine creatinine; U-urea, urine urea. These data are from rats 48 hours after release of BUO without α-MSH treatment (n=5) or with α-MSH treatment (n=6)and time-matched sham-operated control rats (n=4).
*P<0.05 compared with sham-operated control rats
#P<0.05 compared to nontreated rats with 48 hours after release of BUO

Reference List

Alvarez Arroyo, M. V., C. Caramelo , C. M. Angeles, F. R. Gonzalez Pacheco, O. Martin, and J. Arias. Role of vascular endothelial growth factor in the response to vessel injury. *Kidney Int.Suppl* 68: S7-S9, 1998.

Anton, M. J., O. J. Ojeda, B. P. Gonzalez, S. N. Gutierrez, J. C. Palma Nieto, and L. A. Leon Garcia. [Increased levels of erythropoietin in the initial phase of acute myocardial infarction]. *An.Med.Interna* 14: 459-461, 1997.

Ataga, K. I. and E. P. Orringer. Renal abnormalities in sickle cell disease. *Am.J.Hematol.* 63: 205-211, 2000.

Barosi, G. Control of erythropoietin production in man [editorial]. *Haematologica* 78: 77-79, 1993.

Bellinghieri, G., V. Savica, C. De Gregorio, and G. De Gregorio. Use of erythropoietin in ischemic and arrhythmic cardiopathy of hemodialyzed patients. *Contrib.Nephrol.* 106: 135-137, 1994.

Bernaudin, M., H. H. Marti, S. Roussel, D. Divoux, A. Nouvelot, E. T. Mackenzie, and E. Petit. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *J.Cereb.Blood Flow Metab* 19: 643-651, 1999.

Bernaudin, M., A. Bellail, H. H. Marti, A. Yvon, D. Vivien, I. Duchatelle, E. T. Mackenzie, and E. Petit. Neurons and astrocytes express EPO mRNA: oxygen-sensing mechanisms that involve the redox-state of the brain. *Glia* 30: 271-278, 2000.

Besarab, A., W. K. Bolton, J. K. Browne, J. C. Egrie, A. R. Nissenson, D. M. Okamoto, S. J. Schwab, and D. A. Goodkin. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin [see comments]. *N.Engl.J.Med.* 339: 584-590, 1998.

Besarab, A. Iron and cardiac disease in the end-stage renal disease setting. *Am.J.Kidney Dis.* 34: S18-S24, 1999.

Besarab, A., S. Frinak, and J. Yee. An indistinct balance: the safety and efficacy of parenteral iron therapy. *J.Am.Soc.Nephrol.* 10: 2029-2043, 1999.

Bhaktaram, V. J., S. Asirvatham, C. Sebastian, J. A. Pederson, J. R. Stanley, L. R. Pennington, and C. A. Sivaram. Successful management of myocardial infarction during pregnancy in a renal transplant recipient . *Clin.Cardiol.* 21: 447-450, 1998.

Bosio, M. and G. Barosi. Postpheochromocytoma erythropoietin-dependent erythrocytosis. A postischemic mechanism? *Am.J.Pediatr.Hematol.Oncol.* 16: 365-367, 1994.

Brown, K. R., W. Carter, Jr., and G. E. Lombardi. Recombinant erythropoietin overdose. *Am.J.Emerg.Med.* 11: 619-621, 1993.

Cavusoglu, H., A. Kayserilioglu, and M. Terzioglu. The effects of general hypoxic hypoxia and renal ischemia on erythropoietin production. *Arch.Int.Physiol Biochim.* 77: 260-274, 1969.

Cavusoglu, H., A. Kayserilioglu, and M. Terzioglu. The relation of the severity of general hypoxic hypoxia to erythropoietin liberation in partial renal ischemia. *New Istanbul.Contrib.Clin.Sci.* 10: 79-95, 1972.

Chagnac, A., D. Zevin, T. Weinstein, U. Gafter, A. Korzets, and J. Levi. Erythrocytosis associated with renal artery thrombosis in a patient with polycystic kidney disease on hemodialysis. *Acta Haematol.* 84: 40-42, 1990.

Charmes, J. P., T. H. Ton, M. Rince, C. Dupre-Goudable, and D. Durand. [The heart in chronic kidney failure patients]. *Rev.Prat.* 42: 427-431, 1992.

Chen, Y. C., J. C. Yeh, H. S. Chen, and H. C. Hsu. Secondary polycythemia associated with membranous nephropathy. *Clin.Nephrol.* 33: 148-151, 1990.

Conrad, K. P. and D. F. Benyo. Placental cytokines and the pathogenesis of preeclampsia. *Am.J.Reprod.Immunol.* 37: 240-249, 1997.

Crowley, J. P., C. R. Valeri, J. B. Metzger, L. Pono, and J. Chazan. Hemoglobin and 2,3-diphosphoglycerate levels in transfused dialysis patients with myocardial infarction. *Ann.Clin.Lab Sci.* 22: 11-17, 1992.

Del Mastro, L. and M. Venturini. Strategies for the Use of Epoetin Alfa in Breast Cancer Patients. *Oncologist.* 3: 314-318, 1998.

Dougherty, J. E., R. C. Gallagher, J. A. Hirst, M. J. Rinaldi, J. M. Biskup, R. D. Chamberlain, and D. Waters. Coronary stent placement as a bridge to coronary artery bypass surgery in an unstable, anemic Jehovah's Witness patient: a case report and review of bloodless surgery techniques. *Conn.Med.* 61: 195-199, 1997.

Estioko, M. R., R. S. Litwak, and J. H. Rand. Reoperation, emergency and urgent open cardiac surgery in Jehovah's Witnesses. *Chest* 102: 50-53, 1992.

Fernandez, L. M., R. Marcen, J. Villafruela, J. L. Teruel, A. Tato, M. Rivera, and J. Ortuno. Effect of rHuEpo therapy in dialysis patients on endogenous erythropoietin synthesis after renal transplantation. *Nephron* 73: 54-57, 1996.

Fisher, J. W., H. W. Radtke, W. Jubiz, P. K. Nelson, and A. Burdowski. Prostaglandins activation of erythropoietin production and erythroid progenitor cells. *Exp.Hematol.* 8 Suppl 8: 65-89, 1980.

Fisher, J. W., P. K. Nelson, M. Belegu, M. Hagiwara, and B. Beckman. Prostanoid activation of erythropoiesis. *Haematologia (Budap.)* 17: 137-149, 1984.

Forsythe, J. A., B. H. Jiang, N. V. Iyer, F. Agani, S. W. Leung, R. D. Koos, and G. L. Semenza. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol.Cell Biol.* 16: 4604-4613, 1996.

Gombotz, H. Subcutaneous epoetin alfa as an adjunct to autologous blood donation before elective coronary artery bypass graft surgery. *Semin.Hematol.* 33: 69-70, 1996.

Gombotz, H. Subcutaneous epoetin alfa as an adjunct to autologous blood donation before elective coronary artery bypass graft surgery. *Semin.Hematol.* 33: 69-70, 1997.

Gross, D. M., V. M. Mujovic, W. Jubiz, and J. W. Fisher. Enhanced erythropoietin and prostaglandin E production in the dog following renal artery constriction. *Proc.Soc.Exp.Biol.Med.* 151: 498-501, 1976.

Hase, H., Y. Imamura, R. Nakamura, Y. Inishi, K. Machii, and T. Yamaguchi. Effects of rHuEPO therapy on exercise capacity in hemodialysis patients with coronary artery disease. *Jpn.Circ.J.* 57: 131-137, 1993.

Henschel, A., L. Dannenberg, U. Gobel, A. Ott, and U. Schultz-Ehrenburg. [Disseminated ischemic necrosis and livedo racemosa in a chronic dialysis patient with calciphylaxis]. *Hautarzt* 50: 439-444, 1999.

Hogue, C. W., Jr., L. T. Goodnough , and T. G. Monk. Perioperative myocardial ischemic episodes are related to hematocrit level in patients undergoing radical prostatectomy [see comments]. *Transfusion* 38: 924-931, 1998.

Hyde, T. A. The effect of nephrectomy and renal ischaemia on liver regeneration. *J.Pathol.Bacteriol.* 96: 131-136, 1968.

Imamura, Y. and H. Hase. [Effect of recombinant human erythropoietin (rEPO) on angina pectoris in patients with chronic maintenance hemodialysis]. *Nippon Jinzo Gakkai Shi* 33: 403-408, 1991.

Iseki, K., K. Nishime, H. Uehara, K. Tokuyama, S. Toma, K. Yoshihara, T. Kowatari, S. Terukina, A. Osawa, and K. Fukiyama. Increased risk of cardiovascular disease with erythropoietin in chronic dialysis patients [see comments]. *Nephron* 72: 30-36, 1996.

Jones, D. P. Renal metabolism during normoxia, hypoxia, and ischemic injury. *Annu.Rev.Physiol* 48: 33-50, 1986.

Kedar, A., Z. Wajsman, P. Williams, R. Moore, and G. P. Murphy. Paradoxical increase of renal blood flow in anesthetized hypertensive dog treated with indomethacin. *Urology* 14: 256-259, 1979.

Linde, T., B. Wikstrom, L. G. And rsson, and B. G. Danielson. Renal anaemia treatment with recombinant human erythropoietin increases cardiac output in patients with ischaemic heart disease. *Scand.J.Urol.Nephrol.* 30: 115-120, 1996.

Macdougall, I. C., N. P. Lewis, M. J. Saunders, D. L. Cochlin, M. E. Davies, R. D. Hutton, K. A. Fox, G. A. Coles, and J. D. Williams. Long-term cardiorespiratory effects of amelioration of renal anaemia by erythropoietin [published erratum appears in Lancet 1990 Mar 10;335(8689):614]. *Lancet* 335: 489-493, 1990.

Mandinov, L., P. Kaufmann, F. Brunner, and O. M. Hess. [Anemia and heart function]. *Schweiz.Rundsch.Med.Prax.* 86: 1687-1692, 1997.

Mann, J. F. Hypertension and cardiovascular effects--long-term safety and potential long-term benefits of r-HuEPO. *Nephrol.Dial.Transplant.* 10 Suppl 2: 80-84, 1995.

Massetani, R., R. Galli, R. Calabrese, F. Sartucci, P. Rindi, B. L. Severino, and L. Murri. [Status epilepticus in chronically dialyzed patients treated with erythropoietin]. *Riv.Neurol.* 61: 215-218, 1991.

Maxwell, P. H., D. J. Ferguson, L. G. Nicholls, M. H. Johnson, and P. J. Ratcliffe. The interstitial response to renal injury: fibroblast-like cells show phenotypic changes and have reduced potential for erythropoietin gene expression. *Kidney Int.* 52: 715-724, 1997.

Mirand, E. A., G. P. Murphy, G. S. Johnston, and W. W. Scott. Presence of erythropoietin in plasma of non-anemic rats with renal adenocarcinomas. *Proc.Soc.Exp.Biol.Med.* 122: 528-530, 1966.

Moore, L. W., S. O. Smith, R. P. Winsett, S. R. Acchiardo, and A. O. Gaber. Factors affecting erythropoietin production and correction of anemia in kidney transplant recipients. *Clin.Transplant.* 8: 358-364, 1994.

Morishita, E., S. Masuda, M. Nagao, Y. Yasuda, and R. Sasaki. Erythropoietin receptor is expressed in rat hippocampal and cerebral cortical neurons, and erythropoietin prevents in vitro glutamate- induced neuronal death. *Neuroscience* 76: 105-116, 1997.

Murphy, G. P., E. A. Mirand, H. Takita, R. Schoonees, and J. H. Groenewald. The effects of hypoxia and ischemia on erythropoietin and renin release in dogs. *Invest Urol.* 8: 521-525, 1971.

Claims

1. Method for treatment or prevention of a non-ischemia condition in one or more organ(s) or tissue(s) of the urinary tract system including the kidney and the prostata comprising

administration of an effective dosage of α-MSH and/or of an α-MSH equivalent to the individual in need thereof.

2. Method according to any of the preceding claims wherein the organ(s) or tissue is selected from kidney, tubules and glomeruli.

3. Method according to any of the preceding claims wherein the organ(s) or tissue(s) comprises one or more cell types selected from macrophages, the reticulo endothelial system monocytes, neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, T-cells, B-cells, mast cells, and dendritic cells, the method comprising the administration of a dosage of α-MSH and/or of an α-MSH equivalent to the individual in need thereof.

4. Method according to claim 3 wherein the cell type is selected from T-cells, B-cells, and mast cells, .

5. Method according to any of the preceding claims wherein the dosage of α-MSH and/or of an α-MSH equivalent is administered profylactically for preventing a progress of the condition, or of any symptom of the condition.

6. Method according to any of the preceding claims wherein the dosage of α-MSH and/or of an α-MSH equivalent is administered profylactically for preventing the establishment of the condition or of any symptom of the condition.

7. Method according to any of the preceding claims wherein the dosage of α-MSH and/or of an α-MSH equivalent is administered as a single dosage, regular or continued administration, or as a sequential administration.

8. Method according to any of the preceding claims wherein the condition is caused by an anatomic abnormality of the tissue or organ.

9. Method according to any of claims 1-7 wherein the condition is caused by a chemical trauma including drugs.

10. Method according to any of claims 1-7 wherein the condition type is caused by electromagnetic radiation.

11. Method according to any of claims 1-7 wherein the condition is caused by renal and/or ureteric calculi.

12. Method according to any of claims 1-7 wherein condition is caused by an infection infections by by virus, bacteria, fungus and including AIDS virus, bacterial septicemia, systemic fungal infections, Rickettsial diseases, toxic shock syndrome, infectious mononucleosis, cytomegalovirus infection, cambylobacter, salmonella, influenza, poliomyelitis, toxoplasmosis, Lassa Fever, Yellow Fever, billharziose, colibacteria, enterococcer, preteus, klebsiella, pseudomonas, staphylococcus aureus, staphylococcus epidermidis, candida albicans, and tuberculosis.

13. Method according to any of claims 1-7 wherein the condition is caused by a cancer or a by premalignant a disorder of the unrinary tract system including the kidney and the prostata

14.Method according to any of claims 1-7 wherein the condtion is caused by a cancer or a by premalignant disorder having an impact of the unrinary tract system including the kidney and the prostata, such as acute leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, myeloma, metastasing carcinoma of any origin.

15. Method according to any of the preceding claims wherein the organ is selected from the group comprising ureteres, kidney, bladder, urethra and the prostate gland and wherein the tissue(s) is selected from the group of lymphoid tissues, mucosa, epithelium, and endothelium..

16. Method according to any of claims 1-7 wherein the condition is selected from an interstitiel cystitis, cystitis of the mucosa, a microbial cystitis and a non-microbial cystitis

17. Method according to any of claims 1-7 wherein the condition is caused by any disease selected from retroperitoneal fibrosis, lupus erythematosus, polyarteritis nodosa,

scl rodermia, polymyositis, dermatomyositis, rheumatoid arthritis, anaphylaxis, serum sickn ss, h molytic anaemia, allergic agranulocytosis.

18. Method according to any of claims1-7 wherein the condition is caused by diabetes mellitus.

19. Method according to any of claims 1-7 wherein the organ is the kidney and wherein the condition is recognized as renal failure, nephrotic syndrome or complete or partial urnary tract obstruction, postoperative polyuria.

20. Method according to any of the preceding claims wherein the organ is the kidney and the condition is associated with reduced renal function indicated by one or more of the following conditions; reduced reduced renal blood flow, filtration rate, reduced urinary concentrating ability, reduced urinary concentration capacity, reduced or increased urinary electrolyte excretion (such as sodium, potassium, bicarbonate).

21. Method according to any of the preceding claims wherein the condition is associated with dysregulation of one or more renal sodium transporters.

22. Method according to any of claims 20 and 21 wherein the organ is the kidney and condition is characterised by downregulation of one or more renal sodium transporters.

23. Method according to claim 22 wherein the organ is the kidney and the condition is characterised by downregulation of one or more renal sodium transporters of at least 50 %, such as at least 75% compared to non-treatment.

24. Method according to any of claims 21-23 wherein the sodium transporters is selected from the group consisting of Na,K-ATPase, NHE-3, NaPi-2, BSC-1, TSC and ENaC's.

25. Method according to claim 20 wherein the organ is the kidney and the condition is characterised by dysregulation of one or more renal aquaporins.

26. Method according to claim 25 wherein the organ is the kidney and the condition is characterised by downregulation of one or more renal aquaporins including aquaporins selected from aquaporins 1 to 12, preferably aquaporins 1 to 4.

27. Method according to any of the preceding claims wherein the administration is selected from systemic administration; injection into tissue or into a to body cavity including joints; implantation into tissue or into a body cavity; topical application to the skin or to any gastrointestinal surface, or to a mucosal surface including the lining of body cavities.

28. Method according to any of the preceding claims wherein the administration is selected from parenteral adminstration, including intraperitonal administration, intrathecal administration systemic administration, local administration, topical administration, transmucosal administration and transdermal administration and oral administration.

29. Method according to any of the preceding claims wherein the α-MSH equivalent is a substance acting on the α-MSH receptor and/or on the melanocortin receptor.

30. Method according to any of the preceding claims wherein the α-MSH equivalent is a polypeptide having at least 3 amino acids including the following sequence Lys-Pro-Val, such as Gly-Lys-Pro-Val, or the following sequence His-Phe-Arg,

31. Method according to any of the preceding cliams wherein the treatment or prevention further comprises administration of a dosage unit of EPO and/or an EPO equivalent.

32. Method according to claim 31 wherein the combination of α-MSH or α-MSH equivalent with EPO and/or an EPO equivalent has a synergistic effect.

33. Use of α-MSH and/or a equivalent of α-MSH for the preparation of a medicament for treatment or prevention according to any of claims 1-32.

34. Use according to claim 33 for the preparation of a medicament for injection or systemic administration, characterized in that the medicament is in a form suitable for injection or systemic administration, e.g. a solution or a suspension.

35. Use according to claim 33 for the preparation of a medicament for implantation, characterized in that the medicament is in incorporated into a coating of a medico-technical device or is incorporated into the material of the device itself.

36. Use according to claim 33 for the preparation of a medicament for topical application in the form of a powder, paste, ointment, lotion, gel, cream, emulsion, solution, suspension, spray, aerosol, sponge, strip, plaster, or pad.

37. Use according to claim 33 for the preparation of a medicament for oral administration in the form of tablets, sustained release tablets, resoritablets.

38. Use according to claim 33 for the preparation of a medicament for topical application in the form of a preparation suitable for application on mucosa e.g. a suppository, a tampon, a suspension for irrigation, a tablet or troche, a cream or gel or ointment; or for application on urethral mucosa, a bladder insert or an implant.

39. Use according to any of claims 33-38 wherein the α-MSH and/or α-MSH equivalent is present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament.

40 Method for treatment or prevention of an non-ischemia condition in one or more organ(s) or tissue(s) of the urinary tract system including the kidney and the prostata comprising administration of an effective dosage EPO and/or of an EPOequivalent to the individual in need thereof.

41. Method according to claim 40 wherein the organ(s) or tissue is selected from kidney, tubules and glomeruli.

42. Method according to any of claims 40-41wherein the organ(s) or tissue(s) comprises one or more cell types selected from macrophages, the reticulo endothelial system monocytes, neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, T-cells, B-cells, mast cells, and dendritic cells, the method comprising the administration of a dosage EPO and/or of an EPOequivalent to the individual in need thereof.

43. Method according to claim 42 wherein the cell type is selected from T-cells, B-cells, mast cells, .

44. Method according to any of claims 40-43 wherein the dosage EPO and/or of an EPOequivalent is administered profylactically for preventing a progress of the condition, or of any symptom of the condition.

45. Method according to any of claims 40-44 wherein the dosage EPO and/or of an EPOequivalent is administered profylactically for preventing the establishment of the condition or of any symptom of the condition.

46. Method according to any of claims 40-45 preceding claims wherein the dosage EPO and/or of an EPOequivalent is administered as a single dosage, regular or continued administration, or as a sequential administration.

47. Method according to any of claims 40-46 wherein the condition is caused an anatomic abnormality of the tissue or organ.

48 Method according to any of claims 40-46 wherein the condition is caused by a chemical trauma including drugs.

49. Method according to any of claims 40-46 wherein the condition type is caused by electromagnetic radiation.

50. Method according to any of claims 40-46 wherein the condition is caused by renal and/or ureteric calculi.

51. Method according to any of claims 40-46 wherein condition is caused by an infection infections by by virus, bacteria, fungus and including AIDS virus, bacterial septicemia, systemic fungal infections, Rickettsial diseases, toxic shock syndrome, infectious mononucleosis, cytomegalovirus infection, cambylobacter, salmonella, influenza, poliomyelitis, toxoplasmosis, Lassa Fever, Yellow Fever, billharziose, colibacteria, enterococcer, preteus, klebsiella, pseudomonas, staphylococcus aureus, staphylococcus epidermidis, candida albicans, and tuberculosis.

52. Method according to any of claims 40-46 wherein the condition is caused by a cancer or a by premalignant disorder of the unrinary tract system including the kidney and the prostata

53.Method according to any ofclaims 40-46 wherein the condtion is caused by a cancer or a by premalignant disorder having an impact of the unrinary tract system including the kidney and the prostata, such as acute leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, myeloma, metastasing carcinoma of any origin.

54. Method according to any of claims 40-53 wherein the organ is selected from the group comprising ureteres, kidney, bladder, urethra and the prostate gland.

55.Method according to any of claims 40-54 wherein the condition is selected from an interstitiel cystitis, cystitis of the mucosa, a microbial cystitis and a non-microbial cystitis

56. Method according to claim any of claims 40-55 wherein the tissue(s) is selected from the group of lymphoid tissues, mucosa, epithelium, and endothelium.

57. Method according to any of claims 40-46 wherein the condition is caused by any disease selected from retroperitoneal fibrosis, lupus erythematosus, polyarteritis nodosa, sclerodermia, polymyositis, dermatomyositis, rheumatoid arthritis, anaphylaxis, serum sickness, hemolytic anaemia, allergic agranulocytosis.

58. Method according to any of claims 40-46 wherein the condition is caused by diabetes mellitus.

59. Method according to any of claims 40-46 wherein the organ is the kidney and wherein the condition is recognized as renal failure, nephrotic syndrome or complete or partial urnary tract obstruction, postoperative polyuria.

60. Method according to any of claims 40-59 wherein the organ is the kidney and the condition is associated with reduced renal function indicated by one or more of the following conditions; reduced reduced renal blood flow filtration rate, reduced urinary concentrating ability, reduced urinary concentration capacity, reduced or increased urinary electrolyte excretion (such as sodium, potassium, bicarbonate).

61. Method according to any of the preceding claims wherein the condition is associated with dysregulation of one or more renal sodium transporters.

62. Method according to any of claims 60-61 wherein the organ is the kidney and condition is characterised by downregulation of one or more renal sodium transporters.

63. Method according to claim 62 wherein the organ is the kidney and the condition is characterised by downregulation of one or more renal sodium transporters of at least 50 %, such as at least 75% compared to non-treatment.

64. Method according to any of claims 61-63 wherein the sodium transporters is selected from the group consisting of Na,K-ATPase, NHE-3, NaPi-2, BSC-1, TSC and ENaC's.

65. Method according to any of claims 40-60 wherein the organ is the kidney and the condition is characterised by dysregulation of one or more renal aquaporins.

66. Method according to claim 65 wherein the organ is the kidney and the is characterised by downregulation of one or more renal aquaporins including aquaporins selected from aquaporins 1 to 12, preferably aquaporins 1 to 4.

67. Method according to any of 40-66 claims wherein the administration is selected from systemic administration; injection into tissue or into a to body cavity including joints; implantation into tissue or into a body cavity; topical application to the skin or to any gastrointestinal surface, or to a mucosal surface including the lining of body cavities.

68. Method according to any of 40-67 claims wherein the administration is selected from parenteral adminstration, including intraperitonal administration, intrathecal administration systemic administration, local administration, topical administration, transmucosal administration and transdermal administration and oral administration.

69. Method according to any of claims 40-67 wherein the EPO equivalent is a substance acting on the EPO receptor.

70. Use of EPO and/or an EPO equivalent for the preparation for a medicament for treatment or prevention according to any of claims 40-69

71. Use according to claim 70 for the preparation of a medicament for injection or systemic administration, characterized in that the medicament is in a form suitable for injection or systemic administration, e.g. a solution or a suspension.

72. Use according to claim 70 for the preparation of a medicament for implantation, characterized in that the medicament is in incorporated into a coating of a medico-technical device or is incorporated into the material of the device itself.

73. Use according to claim 70 for the preparation of a medicament for topical application in the form of a powder, paste, ointment, lotion, gel, cream, emulsion, solution, suspension, spray, aerosol, sponge, strip, plaster, or pad.

74. Use according to claims 70 for the preparation of a medicament for oral administration in the form of capsules, tablets, sustained release tablets, and resoritablets.

75. Use according to claim 70 for the preparation of a medicament for topical application in the form of a preparation suitable for application on mucosa e.g. a suppository, a tampon, a suspension for irrigation, a tablet or troche, a cream or gel or ointment; or for application on urethral mucosa, a bladder insert or an implant.

76. Use according to any of claims 70-75 wherein the EPOand/or EPO equivalent is present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament.

77. Use according to any of claims 70-76 wherein the dosage of EPO and/or EPO equivalent is non-toxic to a human.

80. Method according to any of claims 1-31 and 42-69 wherein the prevention or treatment is by use of a combination of a EPO and/or EPO equivalent together with a unit dosage of of α-MSH and/or of an α-MSH equivalent and wherein the effect of the combination is higher than the effect obtained with any of the substances administered alone.

81. Method according to claim 81 where the EPO and/or EPO equivalent and the of α-MSH and/or of an α-MSH equivalent is administered independ ntly of each other.

82. Method according to claim 81 wherein the both drugs is administered at lease one within 48 hours, preferably within 24 hours, such as within 12 hours.

83. A pharmaceutical composition comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent together with a suitable pharmaceutical carrier.

84. A pharmaceutical composition according to claim 83 adapted for the use and method according to any of the preceding claims.

85. A pharmaceutical kit comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent together with a suitable pharmaceutical carrier.

86. A pharmaceutical kit according to claim 85 adapted for the use and method according to any of the preceding claims.

Figure 1

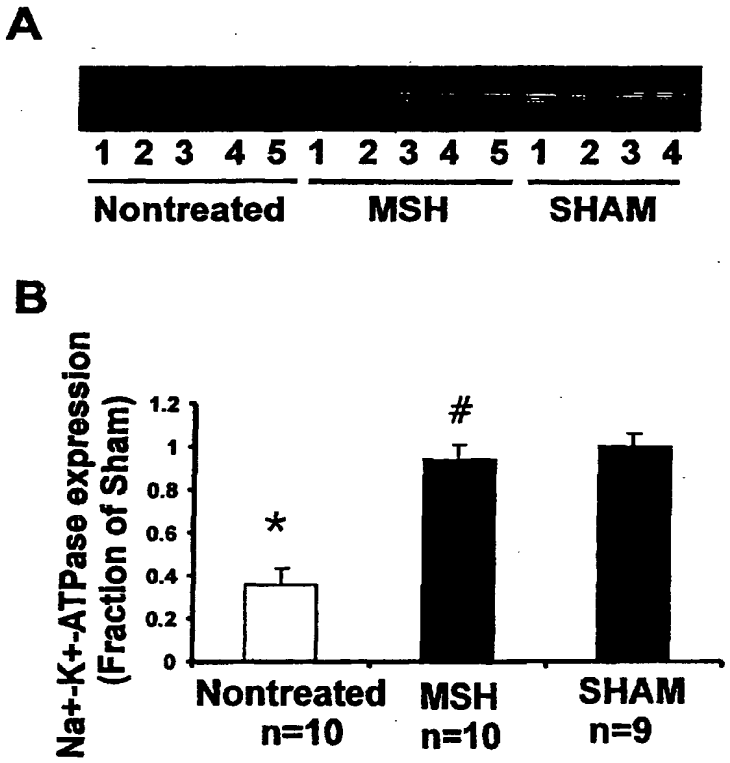


Figure 2

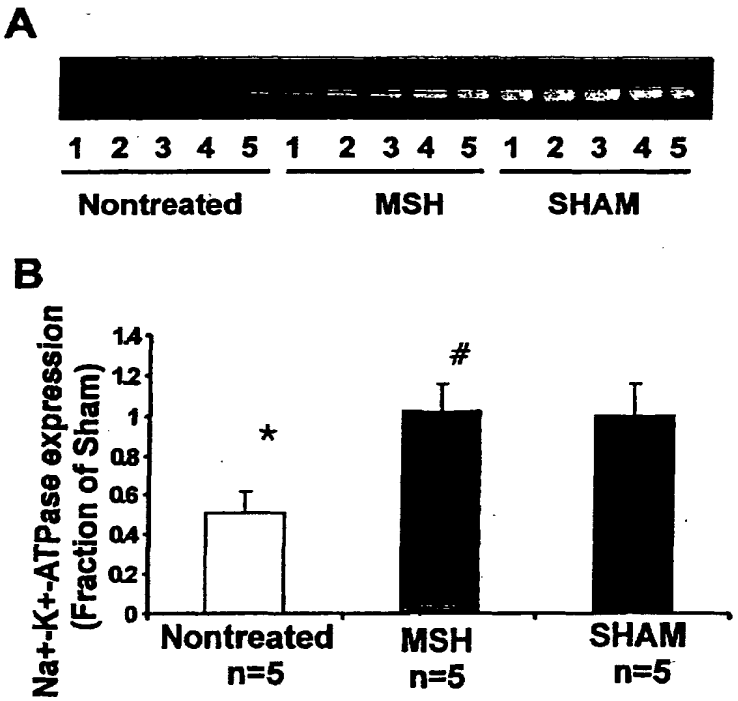


Figure 3

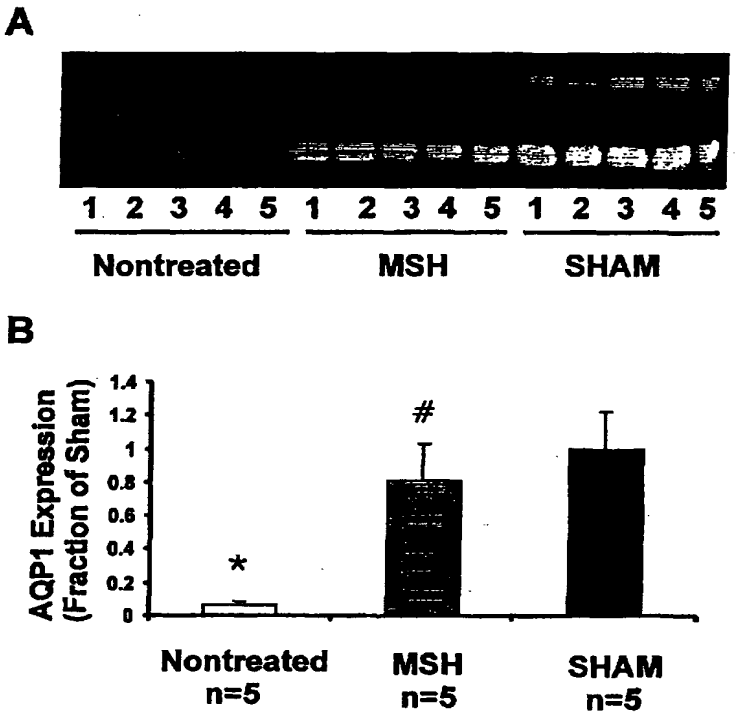


Figure 4

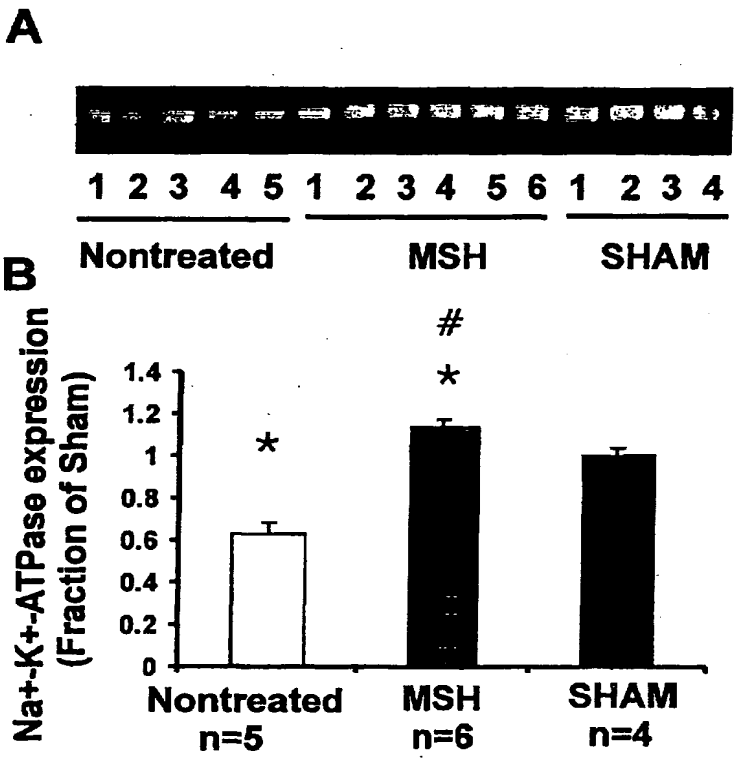
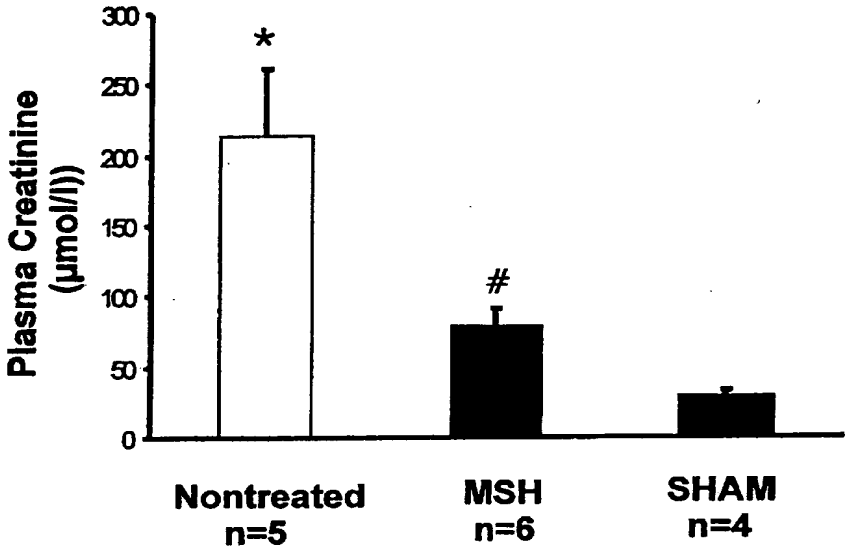
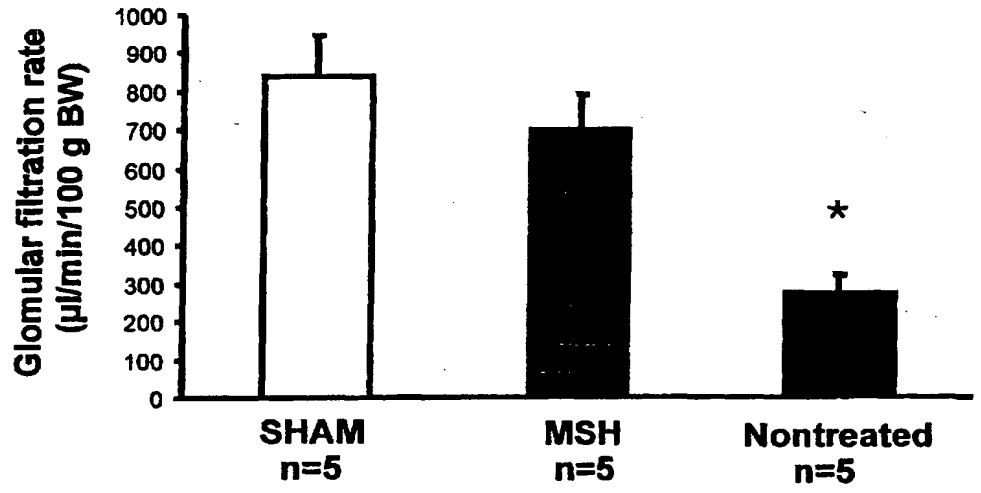


Figure 5



Figur 6

A



B

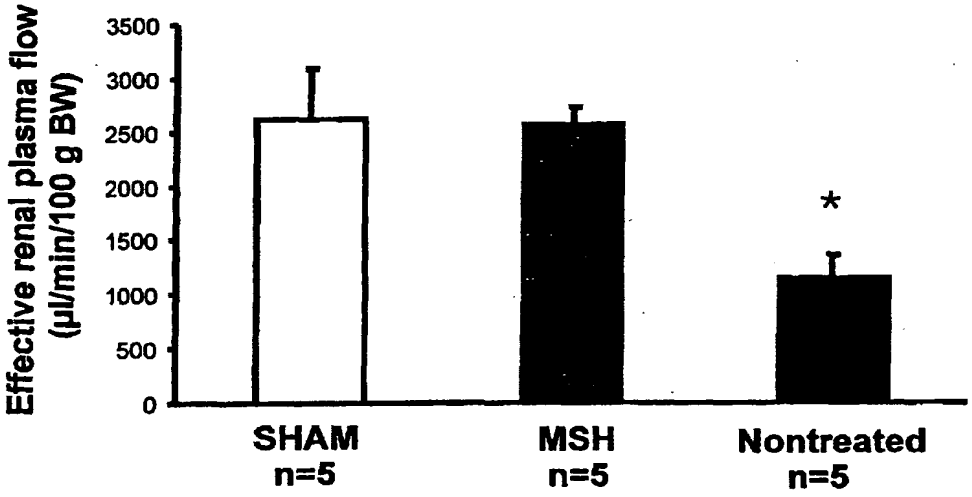
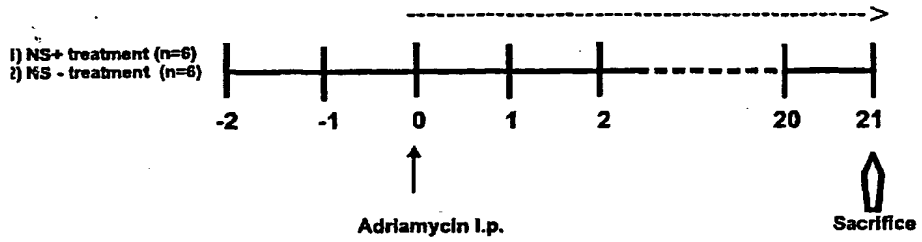


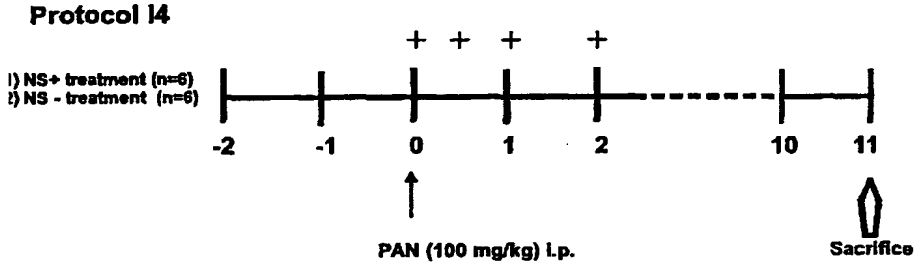
Figure 7

Protocol 13



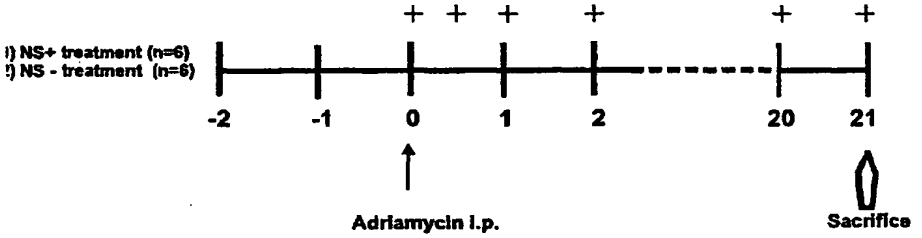
Rats were given [Nle⁴, D-Phe⁷]-alfa-MSH or alfa-MSH (50 ug/day s.c. via osmotic minipumps

Protocol 14



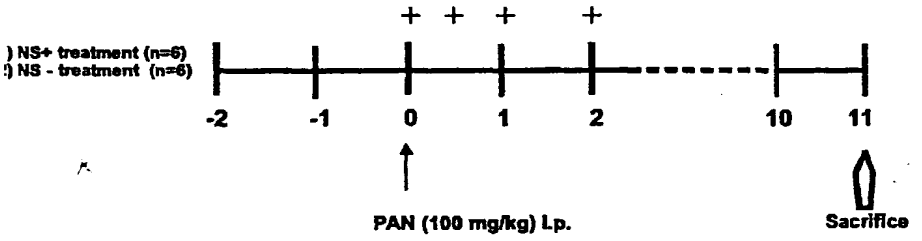
\+ Rats were given [Nle⁴, D-Phe⁷]-alfa-MSH or alfa-MSH (50 ug/I.v.) At day 0 and 6, 24 and 48 hours after aminoglycoside treatment.

Pr t col 15



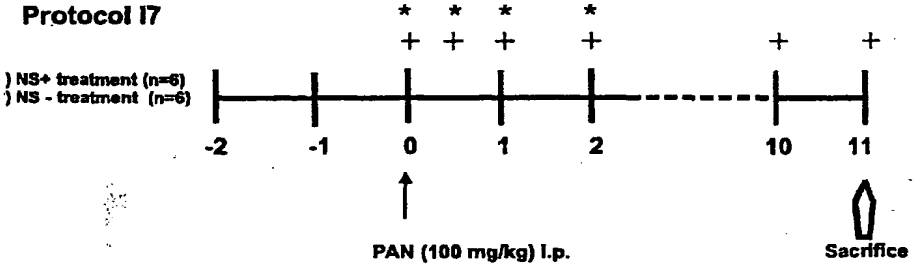
\+ Rats were given epoetin (100 U/kg/day I.p.).

Prot col 16



\+ Rats were given epoetin (100 U/kg/day I.p.).

Protocol 17

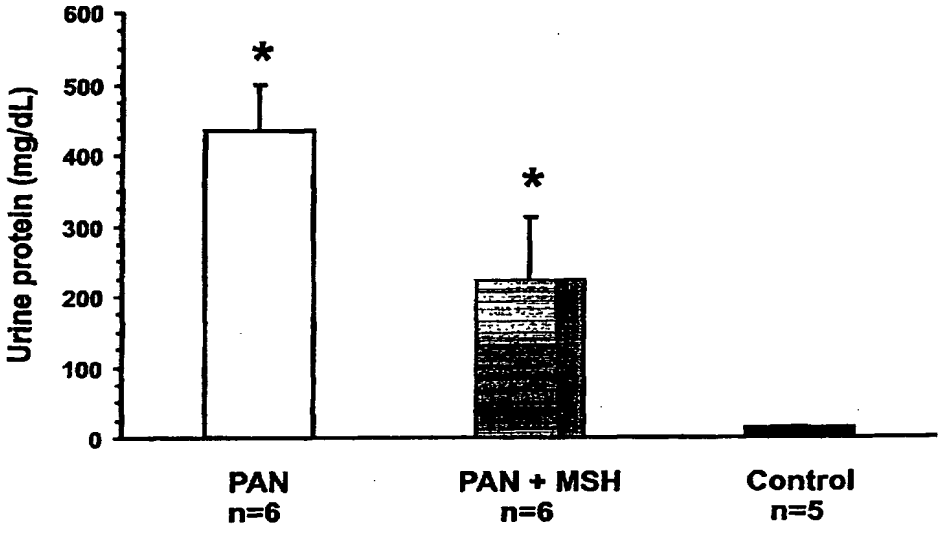


\+ Rats were given epoetin (100 U/kg/day I.p.).

* Rats were given [Nle⁴, D-Phe⁷]-alfa-MSH or alfa-MSH (50 ug/I.v.) At day 0 and 6, 24 and 48 hours after aminoglycoside treatment.

Figure 8

Effect of alpha-MSH on the proteinuria in PAN-induced nephrotic syndrome



*** P<0.06 when rats tretaed with PAN were compared with untreated PAN or with controls**

Figure 9

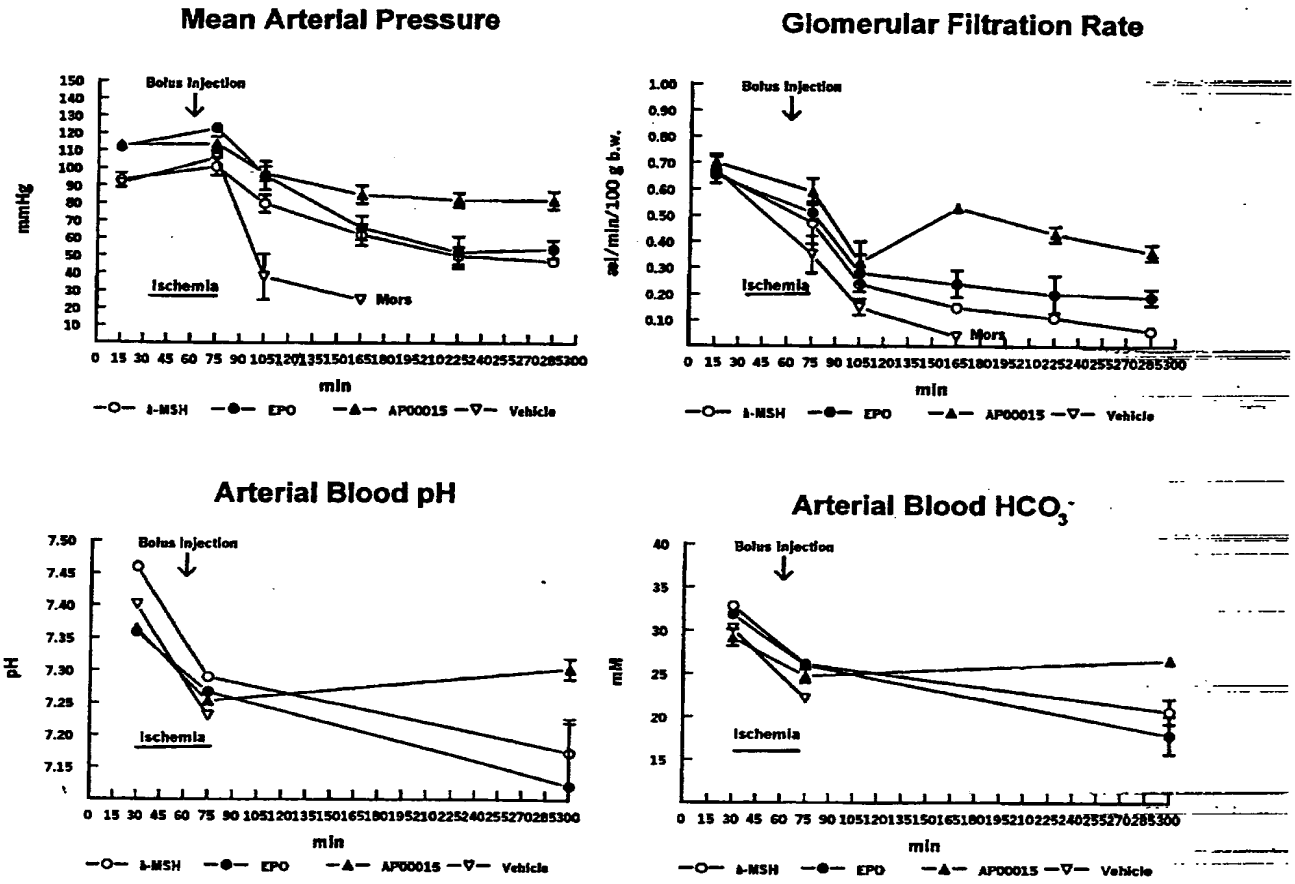


Fig 9